

Development, evaluation and application of Transfersomal Green tea extract (*Camellia sinensis*) formulations

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Abstract

Many studies have shown the beneficial health effects of green tea extract in treatment and protection from many diseases. However, inconsistent results were observed; mainly due to poor absorption and low bioavailability associated with oral administration of green tea extract formulations. Conventional alternative route of administration such as transdermal route is proceeded with an advanced nanoparticle delivery system known as transfersomes. Different formulations of transfersomes are prepared with different types and percentages of edge activators in relation to phospholipid content. This review is intended to evaluate and discuss in-vitro and in-vivo behavior of the prepared transfersomal formulations of green tea extract; which in turn would result in enhancing their therapeutic efficacy.

Keywords— Bioavailability; edge activator; Green tea extract; transdermal and transfersome.

1. Introduction

Since prehistoric times (i.e., for more than 60,000 years) natural products, such as plants, animals, microorganisms, and marine organisms, have been used to alleviate and treat many diseases in medicines [1]. The replacement of normally used drugs with natural products, must of course, have presented a tremendous challenge to humans all over ages. Green tea (*Camellia sinensis*) (fam. Theaceae) is one of the most important natural products that has been used since a long time ago and has been proven safe with many benefits as a source of medicine [2]. The main effective compound in green tea that is responsible for many beneficial effects is a catechin-derived compound, namely, epigallocatechin gallate (EGCG). EGCG has a high antioxidant activity that can provide protection to the body from the risk of cardiovascular diseases, diabetes mellitus, cancer, neurodegenerative disorders, obesity and also exhibits anti-inflammatory effects [3,4].

EGCG is found to be associated with low absorption rate in the alkaline medium as in the small intestine, only less than 5% of EGCG enters the systemic circulation [5]. The unabsorbed EGCG, which does not enter the systemic circulation; will be thrown away to the colon and degraded by intestinal microflora [6].

To overcome these problems, an alternative drug administration route such as transdermal drug delivery system is used, which have the ability to deliver drugs through the skin up to the systemic circulation [7]. This route is characterized by avoidance of gastrointestinal degradation, first pass effect, and drug fluctuations in the blood [8]. To provide optimal antioxidant effects, the bioactive component of green tea (i.e., EGCG) should penetrate into the skin. Furthermore, EGCG is poorly absorbed when administered orally. It is a hydrophilic molecule with a high polarity value ($\log p=0.48$) and a considerable molecular weight (458.37 Da) that becomes an issue in skin penetration [9,10]. In addition, EGCG is known to have high affinity to interact chemically with skin lipid bilayers [11]. In overcoming this, green tea leaves extract can be formulated into a vesicle system or a carrier based lipid (i.e., Transfersomes) to enhance its penetration through skin.

Transfersomes are efficient to deliver natural products, especially for a high molecular weight drugs. This liquefied transdermal transfersomal formulation of green tea leaves can be applied to the skin into a semisolid dosage formulation (i.e., gel), which is often used.

Transfersomes are a hydrophilic ultra-deformable lipid vesicle loaded with active pharmaceutical ingredients (i.e., green tea extract). Each transfersomal preparation consists of phospholipid and an edge activator, which is generally a single chain surfactant with a high radius of curvature. Phospholipid is capable of self-assembling into lipid bilayer, when placed in an aqueous environment, it closes itself to form a vesicle [12,13]. In addition, edge activators are responsible for weakening the vesicles' lipid bilayers, increasing their flexibility and deformability and allowing them to be squeezed through pores of the stratum corneum directly to the systemic circulation. Sodium cholate, sodium deoxycholate, span 60, span 80 and tween 80 are commonly used as edge activators [14–17].

In this study, transfersomes of green tea extract are used for transdermal delivery of EGCG and has furtherly been incorporated in gel for topical application. The aim of this study is to increase in vitro and in vivo penetration of EGCG from green tea leaves extract by formulating them into nanoparticle acceptable new system and thus increases its bioavailability.

2. Materials and methods

2.1 Materials

Green tea leaves extract (*Camellia sinensis*) was purchased from (Andy Biotech Co. Ltd., China with standard potency 98.4 %.) Lecithin and EGCG standard were kindly provided from (Futures manufacture, Egypt) and (Sekem development foundation, Egypt) respectively. A cellophane membrane (Molecular weight cut of 12000–14000) was purchased from United States (USA). The other chemical reagents were analytical grade.

2.2 Methods

2.2.1 HPLC determination of EGCG in Green tea extract

EGCG percentage was accurately measured spectrophotometrically by High performance liquid chromatography (HPLC) in green tea extract against EGCG standard [18] at 278 wave length [19].

2.2.2 Preparation of transfersomes encapsulating Green tea extract

Preparation of Transfersomes

Different transfersomal formulations containing phospholipid (lecithin), cholesterol, and three types of edge activators at different weight ratios (Table 1) were prepared by thin-film hydration technique [20,21].

Characterization of Transfersomes

Determination of entrapment efficiency (EE %): The entrapment efficiencies of transfersomal preparations were determined by HPLC after dispersion in phosphate buffer. The transfersomal dispersion obtained was centrifuged at 10000 rpm. Then, supernatant was assayed for free drug [22].

Morphology and Structure of transfersomes: Morphology characterization of vesicles was performed by transmission electron microscopy (TEM). A drop of the diluted transfersomal suspension was loaded on a carbon-coated copper grid and stained with a drop of aqueous solution of uranyl acetate. The vesicles were air dried before examination [23,24].

Particle size, polydispersity index and zeta potential measurements: Particle size and index polydispersity were measured using a particle size analyzer. The measurements were repeated and the resulting Z-average was selected as the particle size value and measured by photon correlation spectroscopy.

The polydispersity index was conducted to evaluate the particle distribution of transfersomes [24–26]. The zeta potential was measured by dilute drug loaded transfersomal vesicles with distilled water in a cuvette and place a zetasizer probe in it [26–28].

Deformability index test: The elasticity of the transfersomes vesicles was conducted by extrusion method. The transfersomes formulation were extruded through filter membrane using a stainless steel filter holder, pressure was applied and the quantity of vesicles suspension extruded was measured [29–31].

In-vitro drug release studies: The in-vitro permeation behaviour of green tea extract from transfersomal formulations was investigated using diffusion cell model. The diffusion cell was consisted of a cellophane membrane filled with transfersomal preparation, which was placed in a receptor compartment containing phosphate buffer agitated at 100 rpm at $37 \pm 0.5^\circ\text{C}$. An aliquot of 2 mL sample was withdrawn at predetermined time intervals over 24 hours and replaced immediately with an equal volume of fresh diffusion medium. The samples were filtered and analyzed using HPLC [28,32–34].

2.2.3 In-Vivo Study

As transfersomes are difficult to retain at the site of application for sufficient time due to its very low viscosity. In this study, Carbopol had been used to elevate transfersomes' viscosity, so that the prepared transfersomes-based gel showed much better application [35–37]. Transfersomal gel preparations with most suitable required characterization were farther selected to undergo in-vivo study [38].

Animal grouping and administration

Male Wistar rats (250 ± 50 g) were used for the study. The rats were acclimated for at least 7 days in environmentally controlled cages ($23^\circ\text{C} \pm 1^\circ\text{C}$ and 12/12-hour dark/light cycle) with free access to standard food and water and fasted overnight before the experiments. The animals were divided into five groups (ten rats per group):

- GROUP I (control group): received only distilled water
- GROUP II (standard group): received an oral dose of green tea extract using an oral-feeding needle.
- GROUP III: green tea extract loaded gel base was applied on previously shaven dorsal side of rats.
- GROUPS IV and V: selected green tea extract loaded transfersomal gel base (T3 and T6) were applied on previously shaven dorsal side of rats at a dose equivalent to 100 mg/kg body weight, respectively [39].

Animals were sacrificed after daily dosing for 7 days. In the 7th day; at different time points, rats were anaesthetized and RBCs were collected in heparinized tubes from the eyes of rats via the cardio puncture method for serum lipid estimation [40]. Liver from each animal was excised and its homogenate was prepared and centrifuged for 5 min at 4°C. The supernatant obtained was further used for antioxidant assay [38]. Different specimens of the liver, kidney and brain were embedded in paraffin blocks, stained and observed for any changes under light microscope [41].

Assay of total antioxidant capacity (TAC)

The total antioxidant capacity was measured by ferric reducing ability of plasma (FRAP) method. This method is based on the ability of plasma in reducing Fe³⁺ to Fe²⁺ in the presence of TPTZ (2,4,6-tripyridyl-s-triazine) [42].

Determination of catalase activity

The activity of catalase (CAT) was assayed following the method described by Pari and Latha [43].

Estimation of lipid peroxidation

The levels of thiobarbituric acid reactive substance (TBARs) and malondialdehyde (MDA) production were determined by the method described by Draper and Hadley [44].

Lipid profile estimation

Serum HDL (High-density lipoprotein) estimation: determined by the method described by Assmann et al. [45].

Serum cholesterol estimation: determined by the method described by Assmann et al. [45]

Serum LDL (Low-density lipoprotein) estimation: determined by the method described by Assmann et al. [45]

Serum triacylglycerol estimation: determined by the method described by Tietz [46].

Serum VLDL (Very low-density lipoprotein) estimation: determined using the formula, VLDL= total cholesterol – (HDL+LDL).

3. Results and Discussion

3.1 HPLC determination of EGCG in green tea extract

Amount of EGCG presented in green tea extract was measured at 278 wavelengths; this wavelength was advised by Wang et al. [47] and Huo et al. [48].

By optimizing HPLC conditions, the concentration of EGCG in the working green tea extract sample solution was determined (54.24 %) (Figure 1). Calibration curve was constructed to ensure the presence of suitable

amount of EGCG within the extract with further dilutions (Figure 2).

3.2 Preparation of transfersomes encapsulating Green tea extract

Transfersomal preparation encapsulating green tea extract were prepared by conventional rotary evaporation sonication technique in order to remove the organic solvents at a lower temperature [49]. This is mainly due to the highest skin penetrating and bioactive carrying capability, which is frequently associated with such method [50]. In order to optimize the least volume required to dissolve drug, lipid and surfactant uniformly, preliminary screening was performed and combination of ethanol-chloroform in ratio of (1:1) was selected for the next step in the formulation of transfersomes.

A thin lipid film was then generated on the wall of the round bottom flask using rotary evaporator. Phospholipid namely Lecithin, membrane stabilizer (cholesterol) and three edge activators namely span 60, sodium deoxy cholate (SDC) and tween 80, were tried in different ratios to get uniform thin films. Edge activators were added in order to provide flexibility and enhance penetrability through skin [51].

Characteristics of thin film were evaluated by necked eye. It was obvious that films having SDC and spans provided good ranks (better films with uniform thickness) while films having tweens provided bad ranks (thick films with lumps) and this goes with study done by El Zaafarany et al. [20]. In addition, based on the HLB values of surfactants, the affinity for lipids is expected to be in the order of span 60 > Tween 80 > SDC; considering the distribution of surfactant between lipid and aqueous components of transfersomes. This explains the better lipid films associated with using span 60 rather than tween 80 but does not explain the uniform lipid film formation provided by SDC, which may be due to its high solubilizing effect [52]. The ratio of lipid: edge activator (EA) was examined in different ratios (15:1, 7:1 and 3:1) (Figure 3). Avoiding high concentration of edge activator, which will result in bursting of vesicles during storage as mentioned in the study performed by Sheo et al. [30]. Cholesterol was included with lecithin in the prepared transfersomes in a ratio of 4:1; which was advised by Ahad et al. [53]. In addition, it was estimated that the prepared transfersomal vesicles containing cholesterol had a slightly lower particle size than those without cholesterol and increasing cholesterol concentration leads to increase stability and rigidity of the formed transfersomal vesicles. Accordingly, the addition of cholesterol decreases the entrapment efficiency [24].

Effect of edge activator type on EE%

For determination of entrapment efficiency in the formulated transfersomal vesicles, we must first determine the drug content in each preparation. Drug content was found to be in the range of $71.77 \pm 2.72\%$ to $82.25 \pm 0.66\%$, which shows the good capacity of formulation to hold the drug. The maximum drug content was found in formulation T5 (82.25 %) as in (Table 2). While the drug content in preparations using tween 80 showed non-satisfied results in comparison with other preparations. This may be due to high hydrophilic lipophilic balance (HLB) of tween 80 (15) which may cause drug solubilization and diffusion in the aqueous medium during preparation [54]. The EE % values in the performed vesicles ranged from $78.78 \pm 1.89\%$ to $28.61 \pm 2.45\%$ (Table 2). This wide detected range was related to the used edge activator and its concentration. The EE % in case of the formulations containing tween 80 were the lowest, while formulations containing SDC and span 60 showed higher EE% in range of $75.06 \pm 0.53\%$ to $69.33 \pm 0.34\%$ and $78.17 \pm 0.87\%$ to $63.02 \pm 1.65\%$, respectively. Span 60 showed high EE%, which increased non significantly with increasing concentration of span 60, this goes with what have been approved by Abdullh [55], Dubey et al. [56] and Ligan et al. [57]. Also, SDC showed high EE %, which are in agreement with what was

discussed by El Zaafarany et al. [20] and Lopes et al. [58]. The most important factors affecting the entrapment of water-soluble drug molecules in the bilayer vesicles (transfersomes) are the permeability of the biomolecular membranes and the structure continuity of the hydrocarbon chain of the bilayer-forming surfactant [59]. Span 60 has saturated acyl chains, which exists in a gel state at ambient conditions [59]. Such characters render these vesicles less likely to leak the encapsulated water-soluble drug molecules (Green tea extract). As tween 80 is structurally related to span 80, thoughtfully it will undergo the same conformational attitude of span 80, which was confirmed by Abdelkader et al. [60]. However, this did not happen. Tween 80 is fluid at room temperature as well and, in the presence of cholesterol, is disorganized due to the trans-gauche conformations of their acyl chains. This renders tween 80-based transfersomes leakier and permeable to drug than span 60-based transfersomes. These findings explained the lowest EE% achieved by tween 80-based transfersomes [61].

Green tea extract-loaded vesicles prepared using SDC had a significantly higher EE % compared to those prepared by tween 80. This could be attributed to the fact that tween-80 has induced micellar solubilization of extract in the hydration water [20,62], thus decreasing the percentage of drug being entrapped. Similar results were observed by Goindi et al. [63]. By observing the effect of EA ratio on EE %, we can see that whatever EA ratio of span 60 and SDC, the EE% was in high levels, this may be due to that an optimum concentration of the surfactant was reached, which was sufficient to cover the surface of the nanoparticles effectively [64]. Similar results were reported by Lv et al. [65]. While in case of tween 80 when increasing its concentration from 5% to 10%, EE% decreased, which goes in accordance with what has been reported by Venkateswarlu and Manjunath [66], Luo et al. [67] and Salama et al. [68].

Effect of edge activator type on particle size, polydispersity index, zeta potential measurements and deformability index

The transfersomal formulations average sizes were measured and found to be small in the nano-range (86.4 ± 3.61 nm – 209.8 ± 1.32 nm) (Table 2), which in accordance will produce larger surface area (Figures 4,5 and 6) [69]. Particle size distribution of the prepared transfersomal preparations are shown in (Figure 7). Zeta potentials of the transfersomal formulations was found to be negatively charged in the range of -19.65 ± 0.06 mV and -68.24 ± 0.72 mV (Table 2). This charge reveals how tight the drug is being attached to lipid in the formulation (Figure 7). Moreover, improve skin permeation of the drug in transdermal delivery [70].

The transfersomal formulations should be deformable to facilitate its passage through the tiny pores located in the skin [71]. Extrusion measurement was selected to measure the deformability of the prepared formulations and the results were expressed in terms of deformability index in the range of 1.87 ± 0.19 to 4.53 ± 0.28 . (Table 2). The larger the index value of deformability, the transfersomes will be more flexible and penetrate easily through the skin pores despite size of transfersomes itself [72].

The polydispersity index (PDI) describes the degree of non-uniformity of a size distribution of particles [73]. Different size distribution algorithms were observed with the resulting measures (0.19 ± 0.02 - 0.34 ± 0.008) (Table 2).

In vitro drug release studies through cellophane membrane

The initial release should be sufficiently fast to ensure that the therapeutic drug levels are maintained in a timely way in vivo. The afterwards subsequent slow release is mainly due to the slow diffusion of drug molecules through the lipid matrix of the transfersomes [74,75]. T3 and T6 were chosen as the optimized green tea extract transfersomal formulations, which showed lower zeta potential and higher EE %, thereby increasing its ultra-deformability and penetrability [76].

From figure (8), more than 50% of the drug content of all prepared transfersomal formulations was released after 24 hours. Formulation of green tea extract as transfersomal preparations exhibited a controlled release

rate due to the reservoir action of transfersomes. The burst effect over the first 6 hours was obviously observed, followed by a slower release step. This finding could be attributed to the property of the bilayer or transfersomal structure. Moreover, disability of lipid particles of the transfersosomal vesicles to accommodate larger amounts of green tea extract might be another explanation for the burst effect [77–79]. The release rate of green tea extract from the transfersomal formulations was increased gradually with increasing edge activator concentration from ratio of 15:1 to 3:1 with all EAs except for tween 80. Increasing tween 80 concentration in their formulations associated with lower released amount; which may be attributed to their low entrapment efficiency [54]. Therefore, the formulations containing the ratio with optimum release (3:1) with span 60 and SDC were selected for farther in-vivo studies. The percentage drug release after 24 hours for 15:1 (lipid: EA) ratio was 71.25, 74.9 and 69.08 % for span 60, SDC and tween 80 transfersomes, respectively. The percentage drug release after 24 hours for 7:1 (lipid: EA) ratio was 87.89, 88.84 and 60.55 % for span 60, SDC and tween 80 transfersomes, respectively. The percentage drug release after 24 hours for 3:1 (lipid: EA) ratio was 92.02, 91.45 and 57.9 % for span 60, SDC and tween 80 transfersomes, respectively. The differences in molecular ordering caused by EAs may be a reasonable explanation for the observed variations in drug release [80]. Formula T3-containing span 60 in the ratio 3:1 (lipid: EA) exhibited the highest values for release percentage. This may be due to increased solubility of the drug [81] as well as increased fluidity of the lipid bilayer at this EA concentration, which leads to improvement of drug release [20,54].

3.3 In-Vivo Study

The effect of green tea extracts supplementation on ferric reducing ability of plasma (Table 3), explains the variation between the control and the other groups. It can be noticed that the levels of FRAP was increased in groups supplemented with green tea extracts compared to control. Which was higher in groups received it transdermally ($P < 0.0001$ in all 3 groups and 95% CI 0.7915 to 1.1485 in group 4) than the group received it orally with $P = 0.0035$ and 95% CI 0.1758 to 0.7642. Our results are in line with Chacko et al. [82] and Forester and Lambert [83].

In addition, the effect of green tea extracts supplementation on catalase enzyme activity (Table 3), explains the variation between the control and the other groups. It can be noticed that the levels of CAT enzyme activity were influenced in groups supplemented with green tea extracts compared to control group. And in groups (3-5) received it transdermally ($P < 0.0001$ in all 3 groups and 95% CI 2.6387 to 3.3613 in group 5) was better than group received it orally with $P < 0.0001$ and 95% CI 1.5810 to 2.4190. In the present study, we demonstrated that the activity of catalase enzyme is unregulated by catechins, our results are in line with Nelson et al. [84] and Li et al. [85]. However, this was against what was found by Chance et al. [86] and Pal et al. [87], mostly as they used the green tea extract on induced injured animals.

The effect of green tea extracts supplementation on thiobarbituric acid reactive substance (TBARs) and malondialdehyde (MDA) production as in Table (3), explains the variation between the control and the other groups. It can be noticed that the levels of TBARs & MDA levels were lower in groups supplemented with green tea extracts compared to control group. In addition, in groups (3-5) received it transdermally ($P < 0.0001$ in all 3 groups and 95% CI -3.2959 to -1.5041 in-group 3) was lower than group received it orally with $P = 0.0081$ and 95% CI -1.3653 to -0.2347. Mohamadin et al. [88], Awoniyi et al. [89] and Messarah et al. [90] showed results that support our findings.

Changes in plasma lipid profile were observed in different groups (Table 4), cholesterol was decreased by 12% in the group supplemented with green tea extracts orally compared to control group, and by 20%, 10% and 22% in groups (3-5) received it transdermally. HDL level was not affected in the group supplemented with green tea extracts orally compared to control group. However, increased in groups (3-5) received it

transdermally in relation to control group. The Triglycerides, LDL and VLDL levels were decreased in groups supplemented with green tea extracts compared to control group. Our results are consistent with the results of Basu et al. [91], Bornhoeft et al. [92] and Cunha et al. [93]. Whereas Princen et al. [94], Erba et al. [95] and Fukino et al. [96] did not find any effect of green tea-extracted supplementation on serum lipids. Which could be a dose dependent effect.

After oral and transdermal administration of green tea extract, histopathological changes were observed in liver, kidney and brain tissues of rats in all treated groups compared with control group (Figures 9, 10 and 11). Non considerable alterations nor changes were detected in the organs and cells of rats, specimens. This confirms that oral and transdermal administration of green tea extract formulations in such doses for a short time period are not in any other way toxic to the liver, kidney, nor brain of the tested rats.

4. Conclusion

Transfersomes are considered an excellent drug carrier of green tea extract to permeate through skin tissues and so overcome its limitation, which associated with its oral intake. These formulations are found to affect its absorption, cumulative amount, in-vitro penetration of EGCG, in-vivo study, related bioavailability and expected antioxidant activity.

5. Acknowledgments

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6. Declaration of Interest

Authors have no conflict of interest to declare.

7. References

- [1] D.S. Fabricant, N.R. Farnsworth, the value of plants used in traditional medicine for drug discovery., *Environ. Health Perspect.* 109 (2001) 69–75.
- [2] P. Namita, R. Mukesh, K.J. Vijay, *Camellia sinensis* (green tea): a review, *Glob. J. Pharmacol.* 6 (2012) 52–59.
- [3] A.B. Sharangi, Medicinal and therapeutic potentialities of tea (*Camellia sinensis* L.)—A review, *Food Res. Int.* 42 (2009) 529–535.
- [4] J. Anand, B. Upadhyaya, P. Rawat, N. Rai, Biochemical characterization and pharmacognostic evaluation of purified catechins in green tea (*Camellia sinensis*) cultivars of India, *3 Biotech.* 5 (2015) 285–294.
- [5] S. Legeay, M. Rodier, L. Fillon, S. Faure, N. Clere, Epigallocatechin gallate: a review of its beneficial properties to prevent metabolic syndrome, *Nutrients.* 7 (2015) 5443–5468.
- [6] D. Mereles, W. Hunstein, Epigallocatechin-3-gallate (EGCG) for clinical trials: more pitfalls than promises? *Int. J. Mol. Sci.* 12 (2011) 5592–5603.
- [7] E. Anwar, T.D. Utami, D. Ramadon, Transfersomal gel containing green tea (*Camellia sinensis* L. Kuntze) leaves extract: increasing in vitro penetration, *Asian J Pharm Clin Res.* 10 (2017) 294–298.
- [8] D. Ramadon, A.W. Goldie, E. Anwar, Novel transdermal ethosomal gel containing green tea (*Camellia sinensis* L. Kuntze) leaves extract: formulation and in vitro penetration study, *J. Young Pharm.* 9 (2017) 336.

- [9] A. Patel, K. Velikov, Non-covalent interactions of green tea polyphenol: source of novel colloidal structures, in: Green Tea Var. Prod. Health Benefits, Nova Biomedical, 2013: pp. 151–166.
- [10] A. Sharma, S. Saini, A.C. Rana, Transdermal drug delivery system: a review, *Int. J. Res. Pharm. Biomed. Sci.* 4 (2013) 286–292.
- [11] W. Wisuitiprot, A. Somsiri, K. Ingkaninan, N. Waranuch, In vitro human skin permeation and cutaneous metabolism of catechins from green tea extract and green tea extract–loaded chitosan microparticles, *Int. J. Cosmet. Sci.* 33 (2011) 572–579.
- [12] R. Patel, S.K. Singh, S. Singh, N.R. Sheth, R. Gendle, Development and characterization of curcumin loaded transfersome for transdermal delivery, *J. Pharm. Sci. Res.* 1 (2009) 71.
- [13] A. Gupta, G. Aggarwal, S. Singla, R. Arora, Transfersomes: a novel vesicular carrier for enhanced transdermal delivery of sertraline: development, characterization, and performance evaluation, *Sci. Pharm.* 80 (2012) 1061–1080.
- [14] H.A. Benson, Transfersomes for transdermal drug delivery, *Expert Opin. Drug Deliv.* 3 (2006) 727–737.
- [15] P.J. Sarmah, B. Kalita, A.K. Sharma, Transfersomes based transdermal drug delivery: An overview, *Int J Adv Pharm Res.* 4 (2013) 2555–63.
- [16] I. Scognamiglio, D. De Stefano, V. Campani, L. Mayol, R. Carnuccio, G. Fabbrocini, F. Ayala, M.I. LaRotonda, G. De Rosa, Nanocarriers for topical administration of resveratrol: a comparative study, *Int. J. Pharm.* 440 (2013) 179–187.
- [17] Y.S. Elnaggar, W.M. El-Refaie, M.A. El-Massik, O.Y. Abdallah, Lecithin-based nanostructured gels for skin delivery: an update on state of art and recent applications, *J. Controlled Release.* 180 (2014) 10–24.
- [18] K.S. Avadhani, M. Amirthalingam, M.S. Reddy, N. Udupa, S. Mutalik, Development and Validation of RP-HPLC Method for Estimation of Epigallocatechin-3-gallate (EGCG) in Lipid based Nanoformulations, *Res. J. Pharm. Technol.* 9 (2016) 725–730.
- [19] M.C. Roman, J. Hildreth, S. Bannister, Determination of catechins and caffeine in camillia sinensis raw materials, extracts, and dietary supplements by HPLC-uv: single-laboratory validation, *J. AOAC Int.* 96 (2013) 933–941.
- [20] G.M. El Zaafarany, G.A. Awad, S.M. Holayel, N.D. Mortada, Role of edge activators and surface charge in developing ultradeformable vesicles with enhanced skin delivery, *Int. J. Pharm.* 397 (2010) 164–172.
- [21] M.H. Abdallah, Transfersomes as a transdermal drug delivery system for enhancement the antifungal activity of nystatin, *Int J Pharm Pharm Sci.* 5 (2013) 560–7.
- [22] J. Malakar, S.O. Sen, A.K. Nayak, K.K. Sen, Formulation, optimization and evaluation of transferosomal gel for transdermal insulin delivery, *Saudi Pharm. J.* 20 (2012) 355–363.
- [23] J. Guo, Q. Ping, G. Sun, C. Jiao, Lecithin vesicular carriers for transdermal delivery of cyclosporin A, *Int. J. Pharm.* 194 (2000) 201–207.
- [24] S. Duangjit, P. Opanasopit, T. Rojanarata, T. Ngawhirunpat, Evaluation of meloxicam-loaded cationic transfersomes as transdermal drug delivery carriers, *Aaps Pharmscitech.* 14 (2013) 133–140.
- [25] B.V. Mitkari, S.A. Korde, K.R. Mahadik, C.R. Kokare, Formulation and evaluation of topical liposomal gel for fluconazole, *Indian J Pharm Educ Res.* 44 (2010) 324–333.

- [26] D.R. Setyawati, S. Surini, E. Mardiyati, Optimization of luteolin-loaded transfersome using response surface methodology, *Int. J. Appl. Pharm.* 9 (2017) 107–111.
- [27] S. Honary, F. Zahir, Effect of zeta potential on the properties of nano-drug delivery systems-a review (Part 2), *Trop. J. Pharm. Res.* 12 (2013) 265–273.
- [28] J. Shaji, M. Lal, Nanocarriers for targeting in inflammation, *Asian J Pharm Clin Res.* 6 (2013) 3–12.
- [29] S. Mahor, A. Rawat, P.K. Dubey, P.N. Gupta, K. Khatri, A.K. Goyal, S.P. Vyas, Cationic transfersomes based topical genetic vaccine against hepatitis B, *Int. J. Pharm.* 340 (2007) 13–19.
- [30] D.M. Sheo, A. Shweta, C.D. Ram, M. Ghanshyam, K. Girish, K.P. Sunil, Transfersomes-A novel vesicular carrier for enhanced transdermal delivery of stavudine: Development, characterization and performance evaluation, *J. Sci. Specul. Res.* 1 (2010) 30–36.
- [31] T.A. Ahmed, Preparation of transfersomes encapsulating sildenafil aimed for transdermal drug delivery: Plackett–Burman design and characterization, *J. Liposome Res.* 25 (2015) 1–10.
- [32] G.M. El-Mahrouk, O.N. El-Gazayerly, A.A. Aboelwafa, M.S. Taha, Chitosan lactate wafer as a platform for the buccal delivery of tizanidine HCl: in vitro and in vivo performance, *Int. J. Pharm.* 467 (2014) 100–112.
- [33] A.A. Abdelrahman, H.F. Salem, R.A. Khallaf, A.M.A. Ali, Modeling, Optimization, and in Vitro Corneal Permeation of Chitosan-Lomefloxacin HCl Nanosuspension Intended for Ophthalmic Delivery, *J. Pharm. Innov.* 10 (2015) 254–268. <https://doi.org/10.1007/s12247-015-9224-7>.
- [34] D.M. Abdelnabi, M.H. Abdallah, H.A. Elghamry, Buspirone Hydrochloride Loaded In Situ Nanovesicular Gel as an Anxiolytic Nasal Drug Delivery System: In Vitro and Animal Studies, *AAPS PharmSciTech.* 20 (2019) 134. <https://doi.org/10.1208/s12249-018-1211-0>.
- [35] D. Vasudevan, R. Rajan, Effect of permeation enhancers on the penetration mechanism of transfersomal gel of ketoconazole, *J. Adv. Pharm. Technol. Res.* 3 (2012) 112. <https://doi.org/10.4103/2231-4040.97286>.
- [36] R.N. Shamma, I. Elsayed, Transfersomal lyophilized gel of buspirone HCl: formulation, evaluation and statistical optimization., *J. Liposome Res.* 23 (2013) 244–254. <https://doi.org/10.3109/08982104.2013.801489>.
- [37] A. Ahad, M. Raish, A.M. Al-Mohizea, F.I. Al-Jenoobi, M.A. Alam, Enhanced anti-inflammatory activity of carbopol loaded meloxicam nanoethosomes gel., *Int. J. Biol. Macromol.* 67 (2014) 99–104. <https://doi.org/10.1016/j.jbiomac.2014.03.011>.
- [38] J.D. Lambert, D.H. Kim, R. Zheng, C.S. Yang, Transdermal delivery of (-)-epigallocatechin-3-gallate, a green tea polyphenol, in mice, *J. Pharm. Pharmacol.* 58 (2006) 599–604. <https://doi.org/10.1211/jpp.58.5.0004>.
- [39] U.A. Fahmy, Nanoethosomal transdermal delivery of vardenafil for treatment of erectile dysfunction: optimization, characterization, and in vivo evaluation, in: *Drug Des. Devel. Ther.*, 2015. <https://doi.org/10.2147/DDDT.S94615>.
- [40] A. Žabar, V.J. Cvetković, J. Rajković, J. Jović, P.J. Vasiljević, T. Mitrović, Larvicidal activity and in vitro effects of green tea (*Camellia sinensis* L.) water infusion, in: 2013.
- [41] K. Ibrahim, M. Al mutary, A. Bakhiet, H. Khan, Histopathology of the Liver, Kidney, and Spleen of Mice Exposed to Gold Nanoparticles, *Molecules.* 23 (2018) 1848. <https://doi.org/10.3390/molecules23081848>.
- [42] I.F.F. Benzie, J.J. Strain, the ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the

FRAP assay., Anal. Biochem. 239 (1996) 70–76. <https://doi.org/10.1006/abio.1996.0292>.

[43] L. Pari, M. Latha, Protective role of Scoparia dulcis plant extract on brain antioxidant status and lipid peroxidation in streptozotocin diabetic male Wistar rats, in: 2004.

[44] H.H. Draper, M. Hadley, Malondialdehyde determination as index of lipid peroxidation., Methods Enzymol. 186 (1990) 421–431. [https://doi.org/10.1016/0076-6879\(90\)86135-I](https://doi.org/10.1016/0076-6879(90)86135-I).

[45] G. Assmann, H. Schriewer, G. Schmitz, E.O. Hägele, Quantification of high-density-lipoprotein cholesterol by precipitation with phosphotungstic acid/MgCl₂., Clin. Chem. 29 (1983) 2026–2030. <https://doi.org/10.1093/clinchem/29.12.2026>.

[46] N.W. Tietz, Clinical guide to laboratory tests, in: 1983. https://doi.org/10.7326/0003-4819-100-5-774_3.

[47] Z.-M. Wang, Q.-D. Guo, F. Xie, X.-P. Wang, X.-G. Mei, Determination of catechins and caffeine in tea polyphenols by HPLC, Chin. Pharm. J. 46 (2011) 1674–1676.

[48] Q. Huo, J. Hao, R. Shi, Determination of catechin by high performance liquid chromatography and ultraviolet spectrophotometer, Asian J. Chem. 25 (2013) 8940–8942. <https://doi.org/10.14233/ajchem.2013.14923>.

[49] S.D. Maurya, Enhanced transdermal permeation of indinavir sulfate through Stratum Corneum via. Novel permeation enhancers: Ethosome, in: 2010.

[50] G. Cevc, G.D. Blume, A.G. Schätzlein, D. Gebauer, A. Paul, The skin: a pathway for systemic treatment with patches and lipid-based agent carriers, in: 1996. [https://doi.org/10.1016/0169-409X\(95\)00091-K](https://doi.org/10.1016/0169-409X(95)00091-K).

[51] N. Aggarwal, S. Goindi, Preparation and evaluation of antifungal efficacy of griseofulvin loaded deformable membrane vesicles in optimized guinea pig model of Microsporum canis–dermatophytosis., Int. J. Pharm. 437 (2012) 277–287. <https://doi.org/10.1016/j.ijpharm.2012.08.015>.

[52] M.E.Y. Odo, L.C. Cucé, L.M. Odo, A. Natrielli, Action of sodium deoxycholate on subcutaneous human tissue: local and systemic effects., Dermatol. Surg. Off. Publ. Am. Soc. Dermatol. Surg. Al. 33 (2007) 178–88; discussion 188–9. <https://doi.org/10.1111/j.1524-4725.2006.33036.x>.

[53] A. Ahad, A.A. Al-Saleh, A.M. Al-Mohizea, F.I. Al-Jenoobi, M. Raish, A.E.B. Yassin, M.A. Alam, Formulation and characterization of novel soft nanovesicles for enhanced transdermal delivery of eprosartan mesylate, Saudi Pharm. J. SPJ. 25 (2017) 1040–1046. <https://doi.org/10.1016/j.jsps.2017.01.006>.

[54] J.Y. Fang, S.Y. Yu, P.C. Wu, Y.B. Huang, Y.H. Tsai, In vitro skin permeation of estradiol from various proniosome formulations., Int. J. Pharm. 215 (2001) 91–99. [https://doi.org/10.1016/S0378-5173\(00\)00669-4](https://doi.org/10.1016/S0378-5173(00)00669-4).

[55] M.H. Abedullah, PREPARATION AND IN-VITRO EVALUATION OF DICLOFENAC SODIUM NIOSOMAL FORMULATIONS, in: 2013.

[56] A. Dubey, K.B. Bini, J. Kamath, Review on span-60 based non-ionic surfactant vesicles (niosomes) as novel drug delivery, Int J Res Pharm Biomed Sci. 3 (2012) 6–12.

[57] M.A. Lingan, A.H.A. Sathali, M.R.V. Kumar, A. Gokila, FORMULATION AND EVALUATION OF TOPICAL DRUG DELIVERY SYSTEM CONTAINING CLOBETASOL PROPIONATE NIOSOMES, in: 2011.

[58] R. Lopes, C.V. Eleuterio, L. Gonçalves, M. Cruz, A. Almeida, Lipid nanoparticles containing oryzalin for the treatment of leishmaniasis, Eur. J. Pharm. Sci. Off. J. Eur. Fed. Pharm. Sci. 45 (2012) 442–50. <https://doi.org/10.1016/j.ejps.2011.09.017>.

- [59] G. Grant, DRV liposomal bupivacaine: preparation, characterization, and in vivo evaluation in mice, *Pharm. Res.* (n.d.). https://www.academia.edu/24911408/DRV_liposomal_bupivacaine_preparation_characterization_and_in_vivo_evaluation_in_mice (accessed January 24, 2020).
- [60] H. Abdelkader, S. Ismail, A. Kamal, R.G. Alany, Design and evaluation of controlled-release niosomes and discomes for naltrexone hydrochloride ocular delivery, *J. Pharm. Sci.* 100 (2011) 1833–1846.
- [61] T. Yoshioka, B. Sternberg, A.T. Florence, Preparation and properties of vesicles (niosomes) of sorbitan monoesters (Span 20, 40, 60 and 80) and a sorbitan triester (Span 85), *Int. J. Pharm.* 105 (1994) 1–6.
- [62] P. Wavikar, P. Vavia, Nanolipidgel for enhanced skin deposition and improved antifungal activity, *AAPS PharmSciTech.* 14 (2013) 222–233.
- [63] S. Goindi, G. Kumar, N. Kumar, A. Kaur, Development of novel elastic vesicle-based topical formulation of cetirizine dihydrochloride for treatment of atopic dermatitis, *Aaps Pharmscitech.* 14 (2013) 1284–1293.
- [64] R.M. Khalil, A.A. El-Bary, M.A. Kassem, M.M. Ghorab, M. Basha, Influence of formulation parameters on the physicochemical properties of meloxicam-loaded solid lipid nanoparticles, *Egypt. Pharm. J.* 12 (2013) 63.
- [65] Q. Lv, A. Yu, Y. Xi, H. Li, Z. Song, J. Cui, F. Cao, G. Zhai, Development and evaluation of penciclovir-loaded solid lipid nanoparticles for topical delivery, *Int. J. Pharm.* 372 (2009) 191–198.
- [66] V. Venkateswarlu, K. Manjunath, Preparation, characterization and in vitro release kinetics of clozapine solid lipid nanoparticles, *J. Controlled Release.* 95 (2004) 627–638.
- [67] Y. Luo, D. Chen, L. Ren, X. Zhao, J. Qin, Solid lipid nanoparticles for enhancing vinpocetine's oral bioavailability, *J. Controlled Release.* 114 (2006) 53–59.
- [68] H.A. Salama, A.A. Mahmoud, A.O. Kamel, M. Abdel Hady, G.A. Awad, Brain delivery of olanzapine by intranasal administration of transfersomal vesicles, *J. Liposome Res.* 22 (2012) 336–345.
- [69] B. Bhasin, V.Y. Londhe, An overview of transfersomal drug delivery, *Int. J. Pharm. Sci. Res.* 9 (2018) 2175–2184.
- [70] C. Sinico, M. Manconi, M. Peppi, F. Lai, D. Valenti, A.M. Fadda, Liposomes as carriers for dermal delivery of tretinoin: in vitro evaluation of drug permeation and vesicle–skin interaction, *J. Controlled Release.* 103 (2005) 123–136.
- [71] M. Jadupati, G. Amites, N.A. Kumar, Transferosomes: an opportunistic carrier for Transdermal drug delivery system, *Int. J. Pharm. IRJP.* 3 (2012) 35–38.
- [72] H. Chaudhary, K. Kohli, V. Kumar, Nano-transferosomes as a novel carrier for transdermal delivery, *Int. J. Pharm.* 454 (2013) 367–380.
- [73] B. Bera, Nanoporous silicon prepared by vapour phase strain etch and sacrificial technique, *Int. J. Comput. Appl.* 975 (2015) 8887.
- [74] S. Okonogi, T. Oguchi, E. Yonemochi, S. Puttipipatkachorn, K. Yamamoto, Improved dissolution of ofloxacin via solid dispersion, *Int. J. Pharm.* 156 (1997) 175–180.
- [75] A.S. Agro, E.T. Garner, J.W. Wright III, I.C. de Escobar, B. Villeda, M. Seidlin, Clinical trial of ototopical ofloxacin for treatment of chronic suppurative otitis media, *Clin. Ther.* 20 (1998) 744–759.

- [76] P.-S. Wu, Y.-S. Li, Y.-C. Kuo, S.-J.J. Tsai, C.-C. Lin, Preparation and evaluation of novel transfersomes combined with the natural antioxidant resveratrol, *Molecules*. 24 (2019) 600.
- [77] M. NOUNOU, L.K. EL-KHORDAGUI, N.A. KHALAFALLAH, S.A. KHALIL, In vitro drug release of hydrophilic and hydrophobic drug entities from liposomal dispersions and gels, *Acta Pharm.* 56 (2006) 311–324.
- [78] M. Mokhtar, O.A. Sammour, M.A. Hammad, N.A. Megrab, Effect of some formulation parameters on flurbiprofen encapsulation and release rates of niosomes prepared from proniosomes, *Int. J. Pharm.* 361 (2008) 104–111.
- [79] P. Panwar, B. Pandey, P.C. Lakhera, K.P. Singh, Preparation, characterization, and in vitro release study of albendazole-encapsulated nanosize liposomes, *Int. J. Nanomedicine*. 5 (2010) 101.
- [80] H.M. Aboud, A.A. Ali, S.F. El-Menshawe, A.A. Elbary, Nanotransfersomes of carvedilol for intranasal delivery: formulation, characterization and in vivo evaluation, *Drug Deliv.* 23 (2016) 2471–2481.
- [81] R.C. Srivastava, A.N. Nagappa, Application of surface activity in therapeutics, *Stud. Interface Sci.* 21 (2005) 233–293.
- [82] S.M. Chacko, P.T. Thambi, R. Kuttan, I. Nishigaki, Beneficial effects of green tea: a literature review, *Chin. Med.* 5 (2010) 13.
- [83] S.C. Forester, J.D. Lambert, The role of antioxidant versus pro-oxidant effects of green tea polyphenols in cancer prevention, *Mol. Nutr. Food Res.* 55 (2011) 844–854.
- [84] S.K. Nelson, S.K. Bose, G.K. Grunwald, P. Myhill, J.M. McCord, The induction of human superoxide dismutase and catalase in vivo: a fundamentally new approach to antioxidant therapy, *Free Radic. Biol. Med.* 40 (2006) 341–347.
- [85] Y.M. Li, H.Y.E. Chan, Y. Huang, Z.Y. Chen, Green tea catechins upregulate superoxide dismutase and catalase in fruit flies, *Mol. Nutr. Food Res.* 51 (2007) 546–554.
- [86] B. Chance, H. Sies, A. Boveris, Hydroperoxide metabolism in mammalian organs., *Physiol. Rev.* 59 (1979) 527–605. <https://doi.org/10.1152/physrev.1979.59.3.527>.
- [87] S. Pal, S.K. Dey, C. Saha, Inhibition of Catalase by Tea Catechins in Free and Cellular State: A Biophysical Approach, *PLoS ONE*. 9 (2014). <https://doi.org/10.1371/journal.pone.0102460>.
- [88] A.M. Mohamadin, H.A. El-Beshbishy, M.A. El-Mahdy, Green tea extract attenuates cyclosporine A-induced oxidative stress in rats, *Pharmacol. Res.* 51 (2005) 51–57. <https://doi.org/10.1016/j.phrs.2004.04.007>.
- [89] D.O. Awoniyi, Y.G. Aboua, J. Marnewick, N. Brooks, The effects of rooibos (*Aspalathus linearis*), green tea (*Camellia sinensis*) and commercial rooibos and green tea supplements on epididymal sperm in oxidative stress-induced rats, *Phytother. Res. PTR*. 26 (2012) 1231–1239. <https://doi.org/10.1002/ptr.3717>.
- [90] M. Messarah, M. Saoudi, A. Boumendjel, L. Kadeche, M.S. Boulakoud, A. El Feki, Green tea extract alleviates arsenic-induced biochemical toxicity and lipid peroxidation in rats, *Toxicol. Ind. Health*. 29 (2013) 349–359. <https://doi.org/10.1177/0748233711433934>.
- [91] A. Basu, K. Sanchez, M. Leyva, M. Wu, N. Betts, C. Aston, T. Lyons, Green Tea Supplementation Affects Body Weight, Lipids, and Lipid Peroxidation in Obese Subjects with Metabolic Syndrome, *J. Am. Coll. Nutr.* 29 (2010) 31–40. <https://doi.org/10.1080/07315724.2010.10719814>.
- [92] J. Bornhoeft, D. Castaneda, T. Nemoseck, P. Wang, S. Henning, M. Hong, The Protective Effects of Green Tea

Polyphenols: Lipid Profile, Inflammation, and Antioxidant Capacity in Rats Fed an Atherogenic Diet and Dextran Sodium Sulfate, *J. Med. Food.* 15 (2012) 726–32. <https://doi.org/10.1089/jmf.2011.0258>.

[93] C. Cunha, F. Lira, J. Rosa, G. Pimentel, G. Souza, C. Silva, C. Souza, E. Ribeiro, A. Sawaya, C. Nascimento, B. Rodrigues, P. Carvalho, L. Oyama, Green Tea Extract Supplementation Induces the Lipolytic Pathway, Attenuates Obesity, and Reduces Low-Grade Inflammation in Mice Fed a High-Fat Diet, *Mediators Inflamm.* 2013 (2013) 635470. <https://doi.org/10.1155/2013/635470>.

[94] H.M. Princen, W. van Duyvenvoorde, R. Buytenhek, C. Blonk, L.B. Tijburg, J.A. Langius, A.E. Meinders, H. Pijl, No effect of consumption of green and black tea on plasma lipid and antioxidant levels and on LDL oxidation in smokers, *Arterioscler. Thromb. Vasc. Biol.* 18 (1998) 833–841. <https://doi.org/10.1161/01.atv.18.5.833>.

[95] D. Erba, P. Riso, A. Bordoni, P. Foti, P.L. Biagi, G. Testolin, Effectiveness of moderate green tea consumption on antioxidative status and plasma lipid profile in humans, *J. Nutr. Biochem.* 16 (2005) 144–149. <https://doi.org/10.1016/j.jnutbio.2004.11.006>.

[96] Y. Fukino, A. Ikeda, K. Maruyama, N. Aoki, T. Okubo, H. Iso, Randomized controlled trial for an effect of green tea-extract powder supplementation on glucose abnormalities, *Eur. J. Clin. Nutr.* 62 (2008) 953–960. <https://doi.org/10.1038/sj.ejcn.1602806>.

8. Tables

Table (1): Composition of transferosomal vesicles

Formula code	Lecithin (mg)	Cholesterol (mg)	Span 60 (mg)	SDC (mg)	Tween 80 (mg)	Drug (mg)
T1	300	75	25	-	-	100
T2	280	70	50	-	-	100
T3	240	60	100	-	-	100
T4	300	75	-	25	-	100
T5	280	70	-	50	-	100
T6	240	60	-	100	-	100
T7	300	75	-	-	25	100
T8	280	70	-	-	50	100
T9	240	60	-	-	100	100

Table (2): Effect of Edge activator type and percentage on EE %, particle size and charge, polydispersity index and deformability index of the prepared transfersomes

Drug content%	EE%	Particle Size mV	Zeta Potential	Polydispersity index (PDI)	Deformability index
71.96 ± 2.96	67.54 ± 1.99	97.55 ± 1.32	-49.6 ± 0.81	0.21 ± 0.008	3.65 ± 0.13
71.77 ± 2.72	63.02 ± 1.65	86.4 ± 3.61	-68.25 ± 0.72	0.28 ± 0.01	3.14 ± 0.24
82.04 ± 0.79	78.17 ± 0.87	100.1 ± 0.04	-33.15 ± 1.82	0.2 ± 0.004	1.99 ± 0.33
72.63 ± 2.91	69.33 ± 0.34	198.8 ± 2.56	-21.1 ± 0.04	0.34 ± 0.008	2.22 ± 0.09
82.25 ± 0.66	78.78 ± 1.89	170.6 ± 0.49	-19.65 ± 0.06	0.21 ± 0.021	1.87 ± 0.19
79.39 ± 0.07	75.06 ± 0.53	209.8 ± 1.32	-24.95 ± 0.12	0.32 ± 0.005	1.05 ± 0.26
50.46 ± 1.72	43.85 ± 2.34	159.1 ± 3.42	-39 ± 0.64	0.2 ± 0.015	4.53 ± 0.3
33.74 ± 0.13	28.61 ± 2.45	144.5 ± 1.1	-35.75 ± 0.2	0.19 ± 0.02	4.03 ± 0.28
41.57 ± 0.3	38.85 ± 1.51	172.5 ± 1.32	-44.75 ± 0.3	0.23 ± 0.007	3.21 ± 0.22

Table (3): Ferric reducing ability of plasma and changes in activity of catalase and in TBARs & MDA production in different study groups

Groups	FRAP (mg/ml) Mean ± SD	Catalase activity (Units/ml) Mean ± SD	TBARs (nmol/ml) Mean ± SD	MDA (umol/l) Mean ± SD
Control group	1.13 ± 0.19	2.4 ± 0.29	5.04 ± 0.78	3.5 ± 0.21
Standard group	1.65 ± 0.40	4.075 ± 0.56	4.2 ± 0.34	2.4 ± 0.09
Group III	1.9 ± 0.15	5.125 ± 0.26	2.6 ± 1.1	2.18 ± 0.06
Group IV	2.1 ± 0.19	5.075 ± 0.23	3.01 ± 0.34	2.21 ± 0.043
Group V	2.1 ± 0.29	5.425 ± 0.46	3.2 ± 1.01	2.14 ± 0.05

Table (4): Changes in lipid profile in different study groups

Groups	Cholesterol (mg/dl) Mean ± SD	HDL (mg/dl) Mean ± SD	Triglycerides (mg/dl) Mean ± SD	LDL (mg/dl) Mean ± SD	VLDL (mg/dl) Mean ± SD
Control group	110±5	58±3	159±3	60 ± 0.2	10±3
Standard group	91±3	58±3	126±3	50 ± 0.9	12±5
Group III	89±2	64±3	102±5	43 ± 0.9	13±3.2
Group IV	90±2.5	60±2	112±3	35 ± 1	11 ± 3
Group V	86±3	62±1.6	118±2.5	33 ± 1.6	9 ± 4

9. Figures

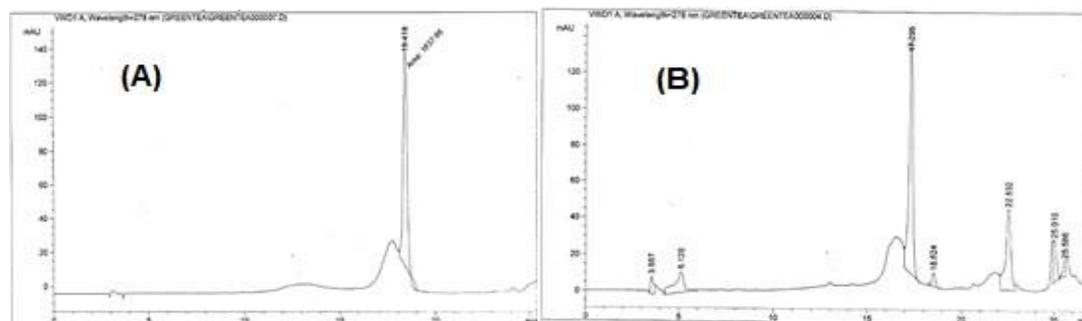


Figure (1): HPLC profile of Green tea extract (a) chromatogram of EGCG standard and (b) chromatogram of green tea extract.

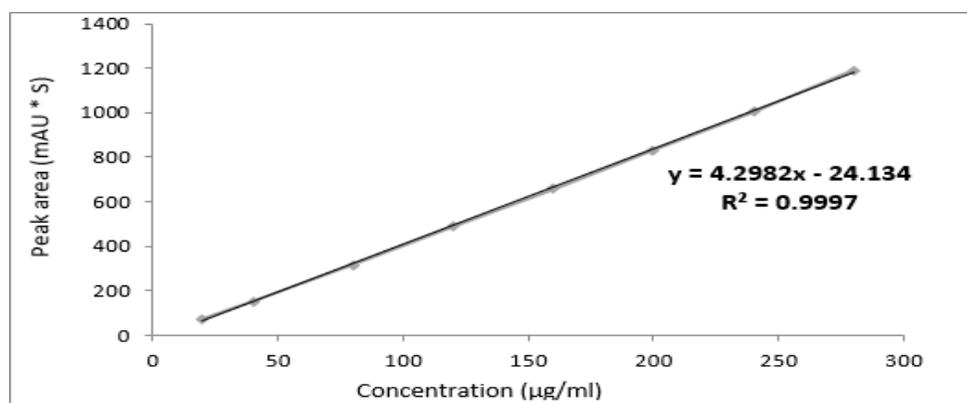


Figure (2): Calibration curve of EGCG in Green tea extract

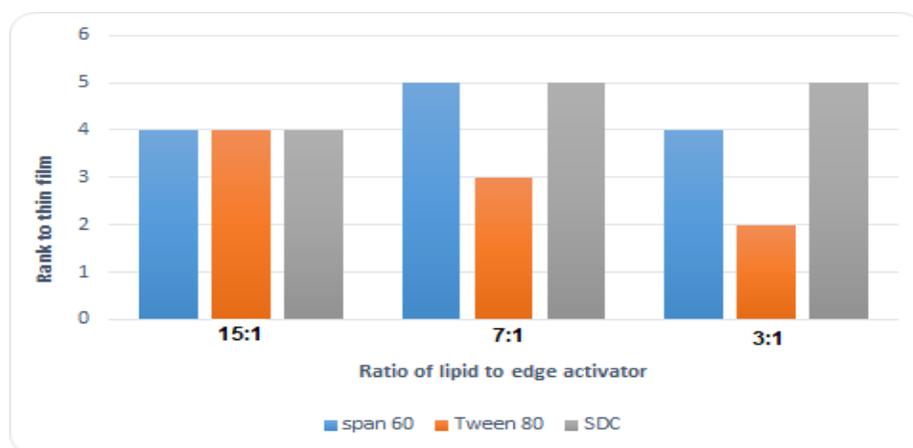


Figure (3) Screening of edge activators by increasing molar ratio in relation to lipids

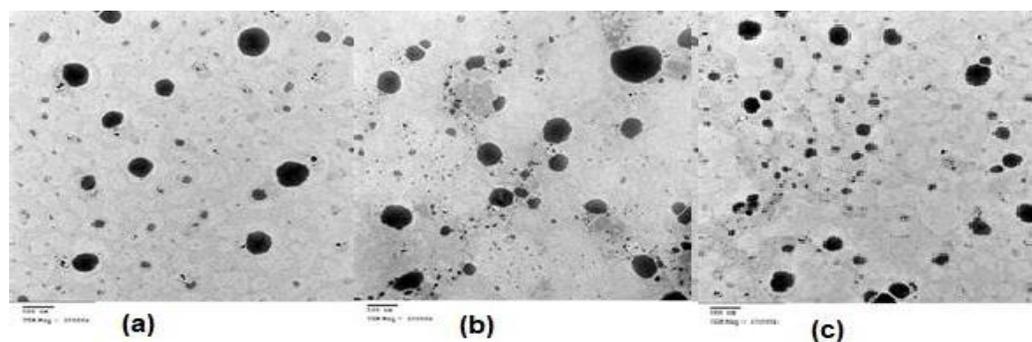


Figure (4) Electron microscopy images of span 60 loaded transfersomal formulations of green tea extract (a) T1 formulation containing (15:1) lipid: span 60 ratios, (b) T2 formulation of (7:1) lipid: span 60 ratios and (c) T3 formulation containing (3:1) lipid: span 60 ratios.

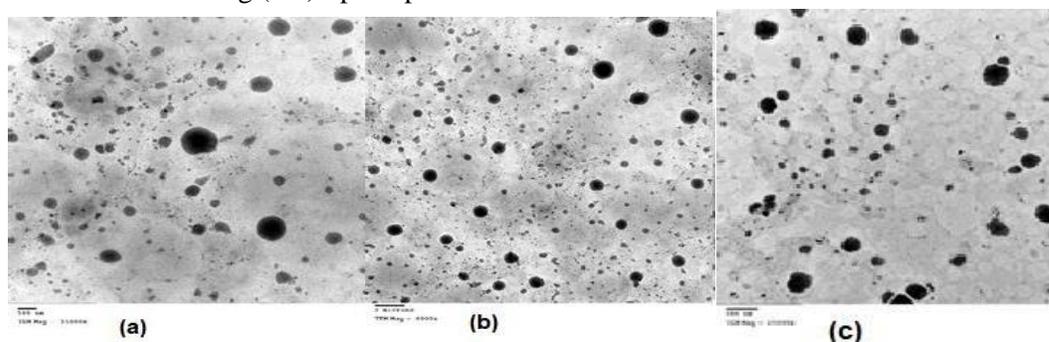


Figure (5) Electron microscopy images of SDC loaded transfersomal formulations of green tea extract (a) T4 formulation containing (15:1) lipid: SDC ratio, (b) T5 formulation of (7:1) lipid: SDC ratio and (c) T6 formulation containing (3:1) lipid: SDC ratio.

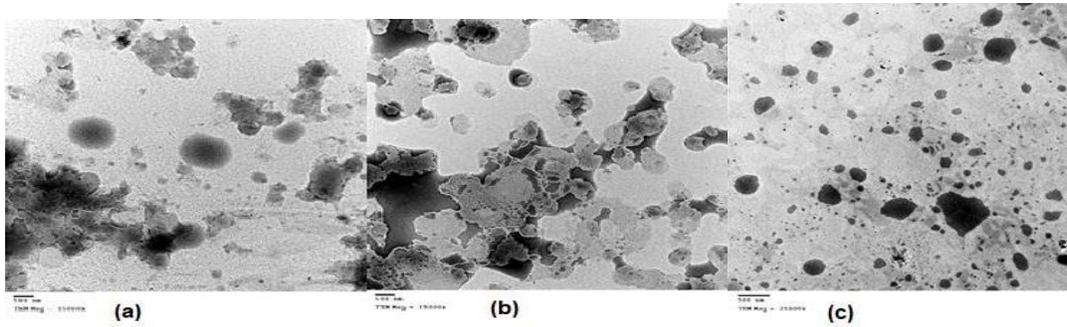
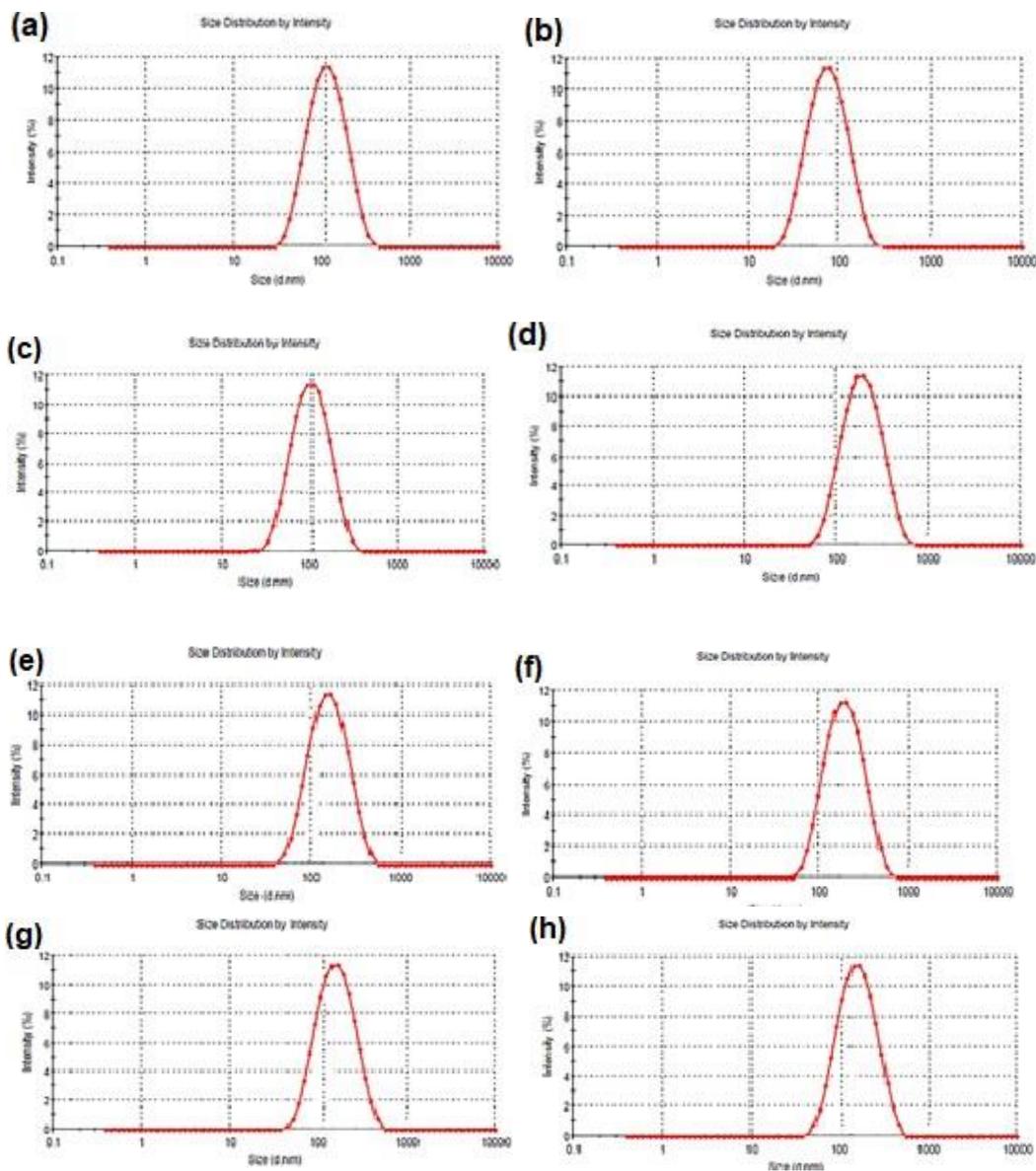


Figure (6) Electron microscopy images of tween 80 loaded transfersomal formulations of green tea extract (a) T7 formulation containing (15:1) lipid: tween 80 ratio, (b) T8 formulation of (7:1) lipid: tween 80 ratio and (c) T9 formulation containing (3:1) lipid: tween 80 ratio.



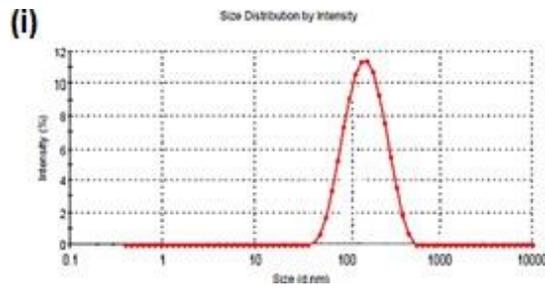


Figure (7) Particle size distribution of (a) Formulation T1, (b) Formulation T2, (c) Formulation T3, (d) Formulation T4, (e) Formulation T5, (f) Formulation T6, (g) Formulation T7, (h) Formulation T8 and (i) Formulation T9

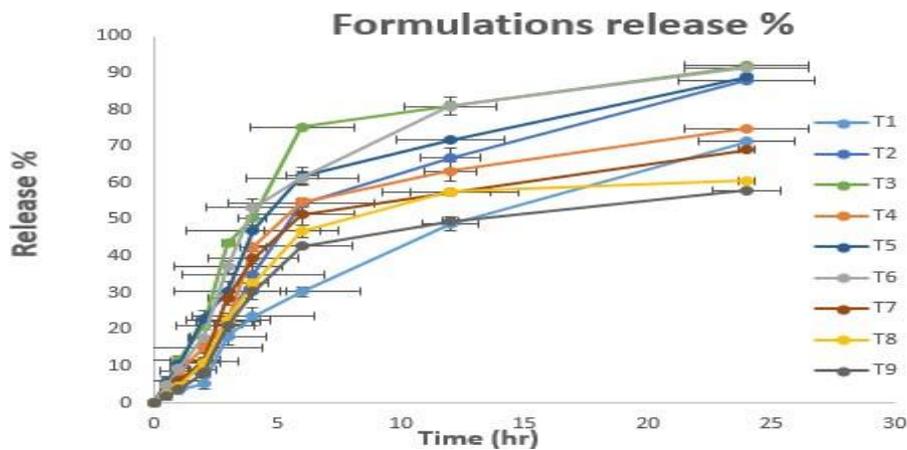


Figure (8) Release profiles of all the prepared transfersomes (each value was an average of three measurements \pm SD)

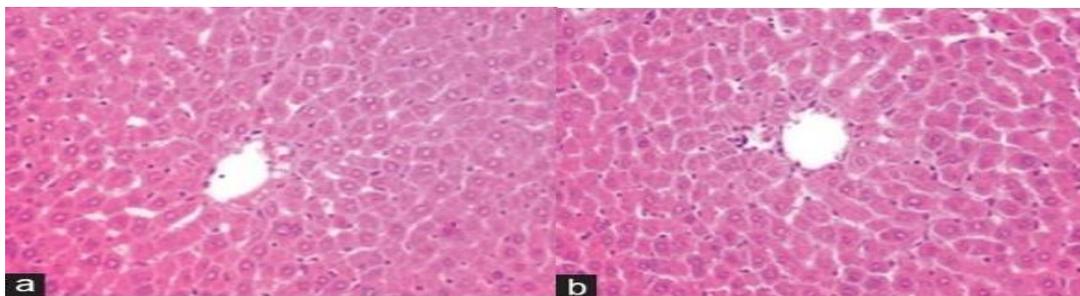


Figure (9): Liver histology of control (A) and experimental rats (B) (H and E, $\times 400$) shows normal hepatic architecture with central vein (CV).

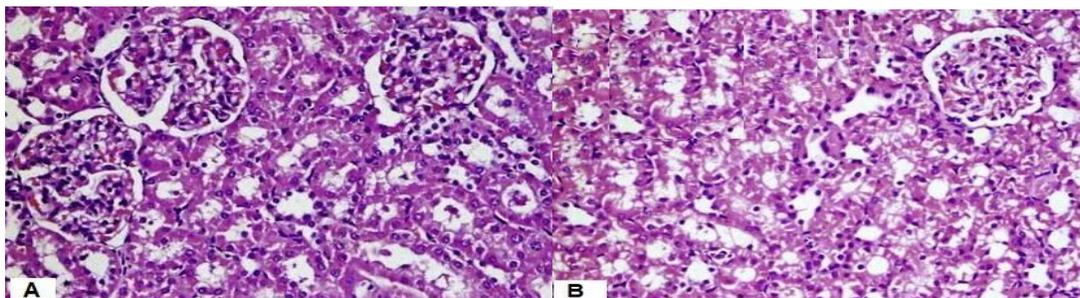


Figure (10): Kidney histology of control (A) and experimental rats (B) (H and E, ×400) shows normal glomerulus and cortical tubules.

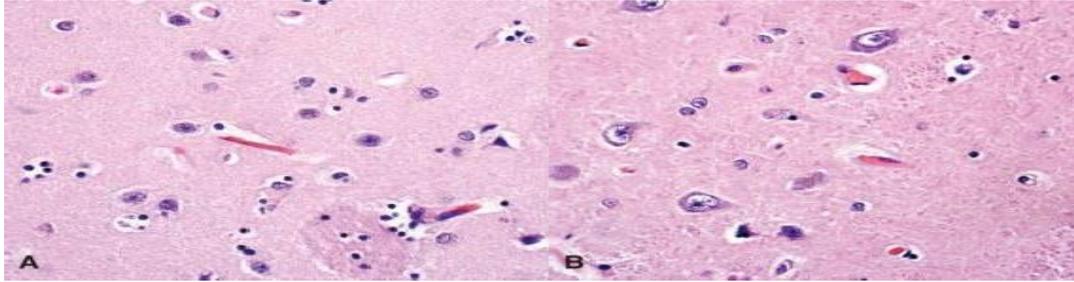


Figure (11): Brain histology of control (A) and experimental rats (B) (H and E, ×400) shows normal variable sized brain tissue and shaped neuronal cells surrounded by eosinophilic neurofibrillary nerve fibers.



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