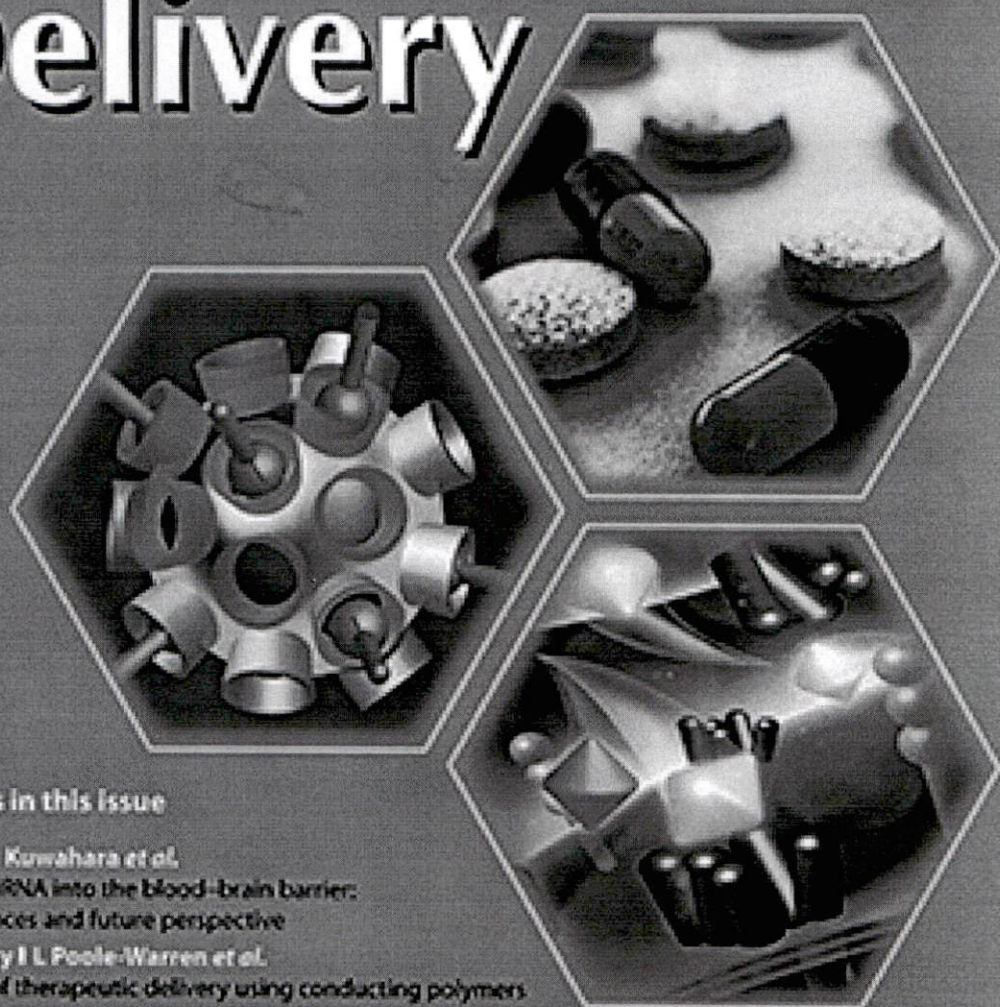


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Therapeutic Delivery



Highlights in this Issue

Editorial | H. Kuwahara *et al.*

Delivery of siRNA into the blood-brain barrier: recent advances and future perspective

Commentary | L. Poole-Warren *et al.*

Challenges of therapeutic delivery using conducting polymers

Research Article | D. Labovitiadi *et al.*

In vitro efficacy of antimicrobial wafers against methicillin-resistant *Staphylococcus aureus*

Review | G. R. Williams *et al.*

Electrospun nanofibers in drug delivery: recent developments and perspectives

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Dr. Ajay K. Banga is a Professor in the Department of Pharmaceutical Sciences at the College of Pharmacy, Mercer University (Atlanta, GA). He serves as Department Chair and as Endowed Chair in transdermal delivery systems. He has served as a consultant to over 25 companies in the area of his research expertise, which is skin delivery systems. Such systems include traditional transdermal patches, dermatologicals, and non-traditional delivery approaches, such as microneedles and iontophoresis, for the delivery of small conventional drugs, macromolecules, and cosmetic actives. Dr. Banga has a Ph.D. in Pharmaceutics from Rutgers University (NJ, USA). He has mentored 40 Ph.D. students as their major advisor, and his laboratory has been funded by over 90 grants/contracts from leading pharmaceutical and cosmetic companies, and by federal funds. Dr. Banga currently serves on the Editorial Board of 10 journals and has served as the Editor-in-Chief for a drug delivery journal. He has written three books in the areas of proteins and transdermal delivery, and published 140 manuscripts, 12 book chapters, and 200 conference abstracts. He has given over 80 invited lectures, and served on over 60 thesis/dissertation advisory committees and as a referee for over 40 journals. He is a Fellow of the American Association of Pharmaceutical Scientists (AAPS).



Brayden D, [University College Dublin](#), Republic of Ireland

Professor in 2014. He was Director of the Science Foundation Ireland (SFI) Research Cluster (The Irish Drug Delivery Research Network) from 2007-2013, is Deputy Coordinator of an FP7 Consortium on oral peptides in nanoparticles ("TRANS-INT", 2012-2017), and is a Co-Principal Investigator in "CURAM", SFI's Centre for Medical Devices (2014-2020). He was made a Fellow of the Controlled Release Society in 2012. He is the author or co-author of more than 200 research publications and patents. Professor Brayden serves on the Editorial Advisory Boards of *Drug Discovery Today*, *European Journal of Pharmaceutical Sciences*, *Advanced Drug Delivery Reviews* and *the Journal of Veterinary Pharmacology and Therapeutics*, and is a Senior Editor of *Therapeutic Delivery*. Professor Brayden works as an independent consultant for drug delivery companies.

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Moses is currently Professor of Pharmacy Practice and Director of the Center for Advancement of Drug Research and Evaluation at the College of Pharmacy, Western University of Health Sciences., in Pomona, California. He was a graduate of the University of California San Francisco. His previous appointments included Professor of Clinical Pharmacy at the University of Connecticut (UCONN), and Professor of Pharmacy and Director of the School of Pharmacy at the Chinese University of Hong Kong (CUHK). At UCONN, his research efforts were devoted primarily to pharmacokinetics and cardiovascular pharmacology and at CUHK, his research team concentrated in pharmacogenetics, drug development of herbal products and sublingual drug delivery. His current research efforts are in translational research and drug development, especially herbal product for cancer. He has supervised/co-supervised over 40 Masters and Ph.D. degree students and post-doctoral fellows. He has authored/co-authored over 300 hundred articles, book chapters, and monographs. His has served as consultant to the Clinical Trial Committee of NHLBI, NIH and USP. He has also served as Advisory Editorial Board member of several pharmaceutical journals including section editor of *Journal of Clinical Pharmacology*. In addition he has served as Secretary and Board member of the American College of Clinical Pharmacology, President of the American Chinese Pharmaceutical Association, and Founding President and Board member of the Asian Association of Schools of Pharmacy. He is a fellow of the American College of Clinical Pharmacy as well as American College of Clinical Pharmacology.

Steve E, [CutisPharma](#), [Wilmington, MA, USA](#)

Steve is currently Executive Vice President and Chief Scientific Officer at CutisPharma. During his tenure in the pharma/biotech industry, he has been involved in building and leading multinational organizations to develop and commercialize small molecule and biologics drug products by applying innovative drug delivery technologies. His work in drug delivery technology innovations and pharmaceutical product development have resulted in over 60 US patent publications, and numerous NDA and ANDA approvals. He also serves on the Board of Advisors in the College of Arts and Sciences at FIU, and provides guidance to entrepreneurship and innovation. He is a Fellow of the American Association of Pharmaceutical Scientists, and a Fellow of the American Institute for Medical and Biological Engineering. Steve received his doctoral degree from the Massachusetts Institute of Technology.

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Lennernäs H, [Uppsala University](#), Sweden

Dr. Hans Lennernäs is a full professor of Biopharmaceutics at Uppsala University since 1 July 2000, Sweden and he also holds an adjunct professor of Biopharmaceutics at Royal Danish School of Pharmacy in Copenhagen, Denmark since 2000. Since 2004 he is also the chief Scientific Office of LIDDS AB (www.liddspharma.com) and the scientific adviser to Duocort AB (www.duocort.com). His research interest is focused on clinical significance of mechanisms and regulation of membrane transport and metabolism of drugs/metabolites in the gastrointestinal tract, hepatobiliary system and cancer tissues. This work is performed in vivo with clinical models in humans and in various tissue and cell culture models. His research aims to develop novel strategies of tissue drug targeting and delivery that aims to improve the clinical use and efficacy of drugs in various disease states, such as metabolic and cancer diseases. Hans Lennernäs has together with gastroenterologists developed and validated two new clinical intestinal perfusion techniques for investigations of intestinal transport and metabolism of drugs and nutrients. He has been the Principal Investigator in an extensive collaboration with Food Drug & Administration, USA, University of Michigan, USA, and Medical Product Agency, Sweden during several years to develop a new guideline for the Biopharmaceutics Classification System. He has established an extensive human permeability database (45 compounds) that today is widely used in academia and pharmaceutical industry. Dr. Lennernäs has been the chairman for numerous international conferences. He serves as reviewer for several scientific journals in clinical pharmacology and pharmaceutical science. His work had led to more 140 publications, 190 invited lectures and more than 300 submitted presentations at scientific meetings. He has supervised 16 doctoral theses and acted as co-supervisor for two neurologists. He has obtained several national and international research grants. Dr. Lennernäs has received Glaxo Wellcome Achievement Award 1997 and Annual Award from the Industrial Pharmacy Section 1998,

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VOLUME 10, ISSUE 7 / JULY 2019

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JULY 2019

Reactive and functionalized electrospun polymeric nanofibers for drug delivery and tissue engineering applications

Hesham M Ismail, Sandi Ali-Adib & Husam M Younes 

Pages: 397-399 | Published Online: 15 July 2019

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Industry News

JUNE 2019

An industry update in therapeutics: what is the latest news?

Catarina P Reis 

Noorma Rosita, Vinta Arnike Meitasari, Mirna Candra Rianti, Dewi Melani Hariyadi & Andang Miatmoko 

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∨ **Preview Abstract**

Research Article

JULY 2019

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Review

JULY 2019

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Enhancing skin penetration of epigallocatechin gallate by modifying partition coefficient using reverse micelle method

Noorma Rosita¹, Vinta Arnike Meitasari¹, Mirna Candra Rianti¹, Dewi Melani Hariyadi¹ & Andang Miatmoko^{*1} 

¹Department of Pharmaceutics, Faculty of Pharmacy, Universitas Airlangga, Kampus C Mulyorejo, Surabaya 60115, Indonesia

*Author for correspondence: andang-m@ff.unair.ac.id

Aim: (-)-Epigallocatechin gallate (EGCG) has been reported as inducing apoptosis in cervical cancer. However, EGCG demonstrates low skin permeability. In order to develop topical delivery of EGCG, the partition coefficient, log P, was modified using a reverse micelle method. **Results & methodology:** During this study, Tween 80 and Span 80 were added to EGCG at hydrophilic-lipophilic balance (HLB) values of 4.3, 6 and 8. The results showed that lowering the HLB value increases the log P value of EGCG and results in higher IC₅₀ values in Henrietta Lacks (HeLa) cancer cells than that of EGCG. Surfactant-modified EGCG-HLB 6 produced faster and deeper skin penetration than EGCG. **Conclusion:** Modification of log P value using a combination of Tween 80 and Span 80 improved cytotoxicity and topical delivery of EGCG.

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Keywords: (-)-epigallocatechin gallate • cytotoxicity • partition coefficient • penetration • Span 80 • Tween 80

Cervical cancer has a high prevalence, constituting the fourth most dangerous form of cancer in women [1]. It has been reported that this form of cancer ranks as the second most common among women in Indonesia, with approximately 18,279 of 32,469 cases proving fatal every year [2]. A recent report confirmed an increase in the number of cervical cancer cases from 702,207 in 2014 to 1,325,776 in 2015 [3].

Cervical cancer is a malignancy that affects the cervix, the lowest part of uterus which protrudes from the top of the vaginal canal [4] and can develop due to infection caused by human papillomavirus. Different forms of treatment for cervical cancer, namely, surgery, radiotherapy, chemotherapy and chemoradiotherapy, are applicable at successive stages of the disease. However, chemoradiotherapy has been known to affect normal cells causing toxic effects to healthy organs or tissues. The development of natural products for use during chemotherapy is currently progressing rapidly due to the large naturally occurring sources of such products. One of the natural compounds that have been proven through research to be an effective component of chemotherapy is epigallocatechin gallate (EGCG) [5,6].

EGCG represents the largest catechin component of green tea (*Camellia sinensis*) and is widely known to be a potent antioxidant. EGCG has been reported as inducing anticancer activity through the mechanism of inhibiting tumorigenesis, proliferation and angiogenesis [7]. EGCG triggers an increase in p53 gene expression in cells experiencing DNA damage [8]; the main function of p53 being to arrest the cell cycle in response to such damage in order that the cell can be repaired before its replication [9]. Moreover, it has been reported that an apoptosis induction occurs in cases of cervical cancer after the administration of EGCG [10,11].

A topical route, such as intravaginal, is proposed as the most appropriate means of drug administration for EGCG in the treatment of cervical cancer. EGCG is highly soluble in water, 40 g/l at 20°C, and demonstrates good stability at pH 3.7 (25°C) and pH 3.9 (40°C) [12]. However, the log P value of EGCG is 1.1 at pH 4.0. It has been reported by Lane *et al.* that the optimal lipophilicity for topical drug penetration is indicated by the log P value within the range of 2–3 [13]. Moreover, it has been reported that EGCG has low cell membrane permeability [14]

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Table 1. Compositions of modified epigallocatechin gallate at various hydrophilic–lipophilic balance values.

HLB value	EGCG (g)	Volume (ml)		
		Tween 80	Span 80	Phosphate buffer pH 5 ± 0.5
8	1	2	4	ad 20
6	1	1	5	ad 20
4.3	1	–	6.0	ad 20
Control	1	–	–	ad 20

EGCG: Epigallocatechin gallate; HLB: Hydrophilic–lipophilic balance.

and is strongly attached to skin tissue, thereby reducing diffusion and the rate of skin permeation [15] due to its polar phenolic group content. Therefore, topical delivery of EGCG in cervical cancer therapy should be extremely limited [10,15]. The use of microemulsion successfully improves topical delivery of EGCG to the deeper skin layer, due to the use of surfactant as emulsifier and oil phase that facilitates EGCG diffusion [16]. Thus, increasing the lipophilicity of EGCG may improve its permeability.

It has been reported that the addition of surfactant to topical drug preparation can enhance the permeability of the drug [17]. Surfactant is an amphiphilic molecule which possesses either hydrophilic head group or lipophilic tail group properties. Surfactants with high hydrophilic–lipophilic balance (HLB) values of 8–18 are more soluble in water and form micelles. On the other hand, surfactants with low HLB values of 3–6 are more soluble in oil and form inverse micelles. Af-idah *et al.* reported that the HLB value of a combination of Span 80 and Tween 80 exerted a significant influence on the physical stability and skin penetration of caffeine microemulsions [18]. In another report, a combination of Span 80 and Tween 80 with an HLB value of 7 increased glutathione lipophilicity from a log P value of -1.4 to one of 2.23, thus improving its skin penetration and resulting in a reduction of MMP-1 [19]. Surfactants can also be used as enhancers capable of changing the nature of the lipid bilayer membranes of stratum corneum, thereby increasing percutaneous permeation [20].

In this study, the permeability of EGCG was improved by increasing the lipophilicity of EGCG through a combination of Span 80 and Tween 80. The modification of EGCG lipophilicity was indicated by a change in the log P value as identified by a partition coefficient test. These modified EGCGs were then evaluated for *in vitro* cytotoxicity in HeLa cells. Moreover, an *in vivo* skin penetration study was evaluated to quantify the increase in EGCG penetration by using Rhodamine-B-labeled micelles. Reverse micelle formation involving a combination of Tween 80 and Span 80 has considerable potential as a means of modifying EGCG lipophilicity.

Materials & methods

Materials

EGCG of 98% purity was purchased from Xi'an Rongsheng Biotechnology Co., Ltd (Xi'an, PR China) and Tween 80 was obtained from Sigma Aldrich (MO, USA). Span 80 was a product from Nanhang Industrial Co., Ltd (Hangzhou, China). Rhodamine-B and n-octanol were secured from Sigma Aldrich. The buffer components were NaH₂PO₄·H₂O and Na₂HPO₄·12H₂O (Merck, Inc., Darmstadt, Germany). All other reagents and solvents used in this study were of the finest available quality.

Preparation of EGCG reverse micelles

EGCG was dissolved in buffer phosphate pH 5 ± 0.5 with a mixture of Tween 80 and Span 80 prepared at different weight ratios, as shown in Table 1, to form systems with HLB values of 8, 6 and 4.3. The HLB system was calculated using the following equation:

$$\% Tween\ 80 = \frac{100(X - HLB_{span\ 80})}{HLB_{Tween\ 80} - HLB_{Span\ 80}}$$

$$\% Span\ 80 = 100 - \% Tween\ 80$$

X refers to the intended HLB value, in other words, 8, 6 and 4.3. The HLB values of Span 80 and Tween 80 are 4.3 and 15, respectively [21].

Determination of equilibrium time for apparent partition coefficient study of EGCG

Approximately 10.0 mg of EGCG were dissolved in 10 ml phosphate buffer pH 5, which had previously been saturated with n-octanol to obtain a 1000 ppm EGCG solution. This was then diluted to produce 40 ppm EGCG solution which was added to n-octanol-saturated buffer at a ratio of 1:10 and mixed in a 50 ml Erlenmeyer flask. The mixture was agitated at a frequency of 150 times per minute using a thermoshaker at $37 \pm 0.5^\circ\text{C}$. The buffer solution was subsequently separated from n-octanol after 5, 10, 15, 30, 45 and 60 min. The samples were centrifuged for 15 min at $811 \times g$. The supernatant was removed and the absorbance of EGCG solution analyzed with an UV spectrophotometer at $\lambda = 271.5 \text{ nm}$. The equilibrium time, defined as the minimum period of agitation required to achieve no significant difference of EGCG levels during the study, was then determined.

Determination of apparent partition coefficient of EGCG after surfactant addition

The determination of apparent partition coefficient was carried out in phosphate buffer pH 5.0 ± 0.05 saturated with n-octanol as the water phase, using a shake flask method [19,22]. The initial concentrations of EGCG were 1000, 2000 and 10,000 ppm for HLB 8, 6 and 4.3, respectively. The EGCG solution was put into a glass vial and added to 1.0 ml of n-octanol as the organic phase, which was saturated with phosphate buffer solution pH 5.0 ± 0.5 . The mixture was then agitated with a thermoshaker at 150 r.p.m. at $37 \pm 0.5^\circ\text{C}$ for 30 min. The water phase was separated from the organic phase by centrifuging samples for 15 min at $811 \times g$. The EGCG level in the water phase was then quantified using a UV spectrophotometer at $\lambda = 271.5 \text{ nm}$.

The log P value was calculated based on the following formula [19]:

$$\text{Log } P = \text{Log} \frac{(C_w^o - C_w^1) \cdot V_w}{C_w^1 \cdot V_o}$$

where P is the apparent partition coefficient; C_w^o is the drug concentration in water phase before equilibrium; C_w^1 is the drug concentration in water phase after equilibrium; V_w is the volume of phosphate buffer as the water phase; and V_o is the volume of n-octanol as the organic phase.

In vitro cytotoxicity study on HeLa cells

HeLa cells were obtained from Laboratory for Joint Basic Experiments, Faculty of Pharmacy, Universitas Airlangga (Surabaya, Indonesia). HeLa cells were cultured in the Rosewell Park Memorial Institute 1640 medium with 10% heat-inactivated fetal bovine serum, penicillin (100 units/ml), streptomycin (100 $\mu\text{g}/\text{ml}$) and amphotericin B (2.5 $\mu\text{g}/\text{ml}$) in a humidified atmosphere containing 5% CO_2 at 37°C . For the *in vitro* cytotoxic assay, HeLa cells were seeded separately at a density of 5×10^3 cells per well in 96-well plates and maintained in the medium for 24 h before treatment.

To examine the cytotoxicity, the cells were treated with medium containing EGCG at concentrations of 10, 20, 40, 80 and 100 ppm in EGCG, surfactant-modified EGCG-HLB 4.3, or surfactant-modified EGCG-HLB 6. To aid sample solubility in medium, surfactant-modified EGCG was added to DMSO. The cells were then incubated for 48 h. After treatment, the cell number was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. On conclusion of the culture media incubation period, the medium was discarded and replaced with 100 μl of 5% 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide solution (Sigma Aldrich) in each well. Thereafter, the plate was placed in an incubator for 4 h, until the formation of formazan crystals was observed under a light microscope. Then, a 100 μl of 10% sodium dodecyl sulfate solution in 0.01 M HCl was subsequently being added to each well. The plate was wrapped in aluminum foil, incubated overnight in the dark at room temperature and its absorbance measured with an ELISA reader at $\lambda = 595 \text{ nm}$. Cell viability was expressed relative to the absorbance of untreated cells and media, while the concentration leading to 50% cell viability (IC_{50}) was also calculated.

In vivo skin penetration study

For the purposes of the *in vivo* skin penetration study, Rhodamine-B-labeled micelle was prepared to enable evaluation of the ability of surfactant-modified EGCG to permeate the skin of rats. Rhodamine-B was initially dissolved with 10 ml phosphate buffer pH 5 to produce a 0.01% Rhodamine-B solution. Approximately 1 g of EGCG was then added and the mixture stirred until homogenous with roughly 1 ml of Tween 80 and 5 ml of Span 80 being introduced. Phosphate buffer pH 5 was added to produce a final mixture with a volume of 20 ml which

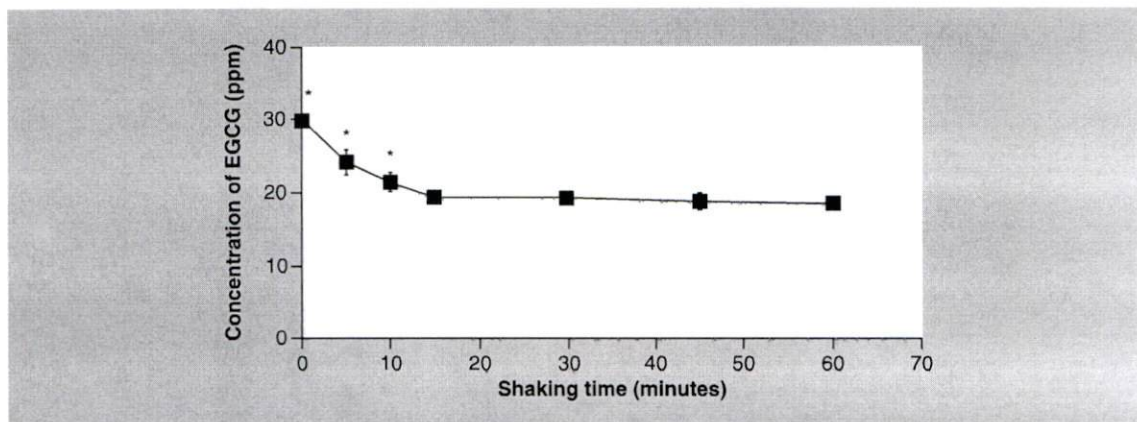


Figure 1. The relation between shaking time (minutes) and epigallocatechin gallate concentration (ppm) observed in a mixture of *n*-octanol and 0.01 M phosphate buffer pH 5 ± 0.05 at temperature of $37 \pm 0.5^\circ\text{C}$. All data were in three replicates.

* $p < 0.05$ versus 60 min.

was then stirred at 1000 r.p.m. for 15 min. The mixtures of Rhodamine-B and surfactant and Rhodamine-B, which had been freeze-dried to produce a sample that had semi-solid consistency, and EGCG were used as the control groups.

The research subjects were Wistar rats (male: 8–10 weeks old and 150–200 g in weight) purchased from the Animal Laboratory, Faculty of Pharmacy, Universitas Airlangga (Surabaya, Indonesia). All subjects were maintained and treated in conditions which complied with the guiding principles for the care and use of laboratory animals as established by Faculty of Veterinary Science, Universitas Airlangga, Indonesia.

The subjects were divided into three groups each containing four subjects, representing surfactant, EGCG, and surfactant-modified EGCG HLB 6 treatment for Group 1, Group 2 and Group 3, respectively. The samples were applied non-occlusively to a 6 cm^2 area of the abdominal skin of subjects. To avoid sample spilled out from the designated area, the rat's abdomen was covered with a water-impermeable plastic paper that had a hole created at an exact size of $2 \times 3\text{ cm}^2$ for sample application. The dose was equal to 50 mg of EGCG per kg. During the study, all subjects were anesthetized with an intramuscular injection of Ketamine (20 mg/kg body weight). After 2 h of drug administration, the subjects were sacrificed by means of cervical dislocation. The section of skin tissue was then excised and embedded in an optimal cutting temperature (OCT) compound (Tissue Tek[®], Sakura Finetechnical Co., Tokyo, Japan). It was then processed by frozen sectioning of $5\ \mu\text{m}$ thickness. The prepared skin tissue slices were then observed under a fluorescence microscope (FSX100, Olympus, Tokyo, Japan).

Statistical analysis

All data were in the form of 3–4 replicates and presented as the mean \pm SD. To evaluate the significance of the difference, the data were analyzed by one way ANOVA followed by least significant difference test with p -value < 0.05 and < 0.01 using SPSS Software v.21.0.

Results & discussion

Determination of equilibrium time & apparent partition coefficient of EGCG

To increase the skin penetration of EGCG for topical drug delivery, the modification of EGCG hydrophobicity was investigated by reverse micelle method. First, the equilibrium time required for evaluating apparent partition coefficient or $\log P$ of EGCG and surfactant-modified EGCG was determined. It has been reported that EGCG is stable in low pH condition, in other words, pH 2–5.5 and it is unstable in alkaline condition [24]. In addition, for the acceptability of topical vaginal product, the pH should fall within 4–6 [23]. Therefore, in this study, we prepared EGCG in phosphate buffer pH 5. The equilibrium time of EGCG in a phosphate buffer pH 5/*n*-octanol at $37 \pm 0.5^\circ\text{C}$ was reached after constant EGCG levels had been obtained. As shown in Figure 1, the EGCG concentrations at 15, 30, 35 and 60 min were not significantly different. Consequently, it can be postulated that

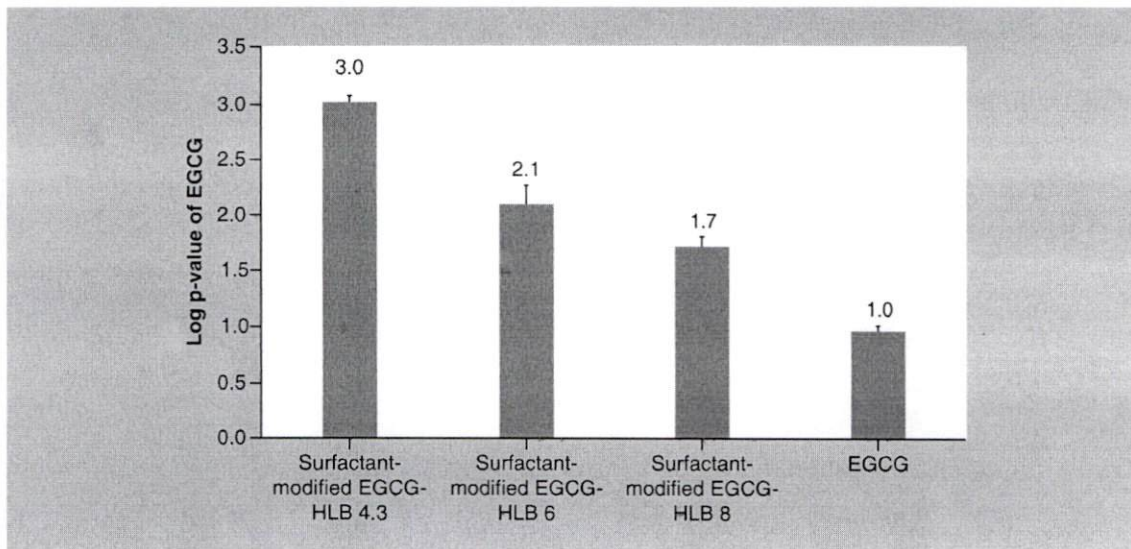


Figure 2. The log P values of epigallocatechin gallate and surfactant-modified epigallocatechin gallate-hydrophilic-lipophilic balance 4.3, 6 and 8. The study was performed by using a mixture of n-octanol and 0.01 M phosphate buffer pH 5 ± 0.05 at $37 \pm 0.5^\circ\text{C}$ and agitating in a thermoshaker for 30 min. All data were in three replicates.

after 15 min the EGCG levels were constant. During this study, 30 min was chosen as the equilibrium time for further experiments.

Determination of the EGCG partition coefficient at pH 5.0 ± 0.5

HLB represents the proportion of hydrophilic and lipophilic nature of surfactants [21]. On the other hand, cancer cells, in addition to other eukaryote cells, possess a membrane consisting of lipoprotein that requires a drug to exhibit similar lipophilicity in permeating it [25]. Thus, preparing drug-surfactant mixture of different HLB values may modify the hydrophobicity of EGCG. The success with which the combination of Span 80 and Tween 80 altered the apparent partition coefficient of EGCG in phosphate buffer pH 5/n-octanol media was investigated. Determination of the apparent partition coefficient ($\log P_{app}$) was conducted by agitating samples equal to 40 ppm EGCG in 0.01 M phosphate buffer solution pH 5.0 as the water phase and n-octanol as the oil phase at $37 \pm 0.5^\circ\text{C}$ for 30 min. It can be seen in Figure 2, that an increasing HLB value reduced the log P value of EGCG. The log P value obtained was subsequently used to determine the selected formula for the study of *in vivo* skin penetration. From the results, it can be proposed that modifying EGCG in the HLB values of 4.3 and 6 produced log P values between 2 and 3, that are similar to the log P value of skin. This may be due to the entrapment of EGCG, that is highly soluble in water, in the inner core of the reverse micelle which probably of the water in oil (w/o) micellar type. This condition creates 'like dissolve like' phenomena between EGCG and more lipophilic components, in other words, n-octanol, in the oil phase [26]. It has been known that Span 80 and Tween 80, which act as the surfactants, contain hydrophilic and lipophilic groups [27] that may facilitate the interaction between EGCG and n-octanol. Therefore, it renders the nature properties of EGCG more hydrophobic.

The cytotoxicity of EGCG on HeLa cells

It has been reported that most EGCG cellular activity is related to its very high concentration of hundreds of micromoles [6,14], which indicates that its polar phenolic hydroxyl group limits EGCG permeability across cell membrane. Improving the hydrophobicity of the system by, for example, using liposom as fusogenic lipid carrier, improved the membrane permeability of EGCG, thus producing effective cellular activity of EGCG at low concentrations [14].

During this study, the log P value was modified by using surfactant combinations of Span 80–Tween 80 prepared at different HLB values. This constituted an effort to increase the lipophilicity of EGCG [28], which was evaluated by phosphate buffer pH 5/n-octanol partition study, in order to improve the membrane permeability and cytotoxicity

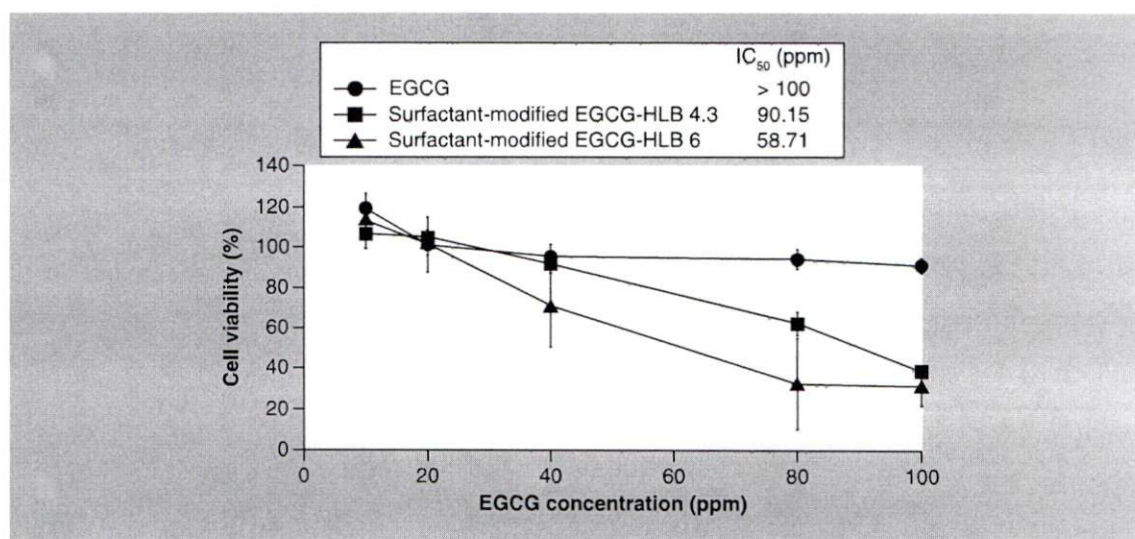


Figure 3. *In vitro* cytotoxicities of epigallocatechin gallate, surfactant-modified epigallocatechin gallate-hydrophilic-lipophilic balance 4.3 and surfactant-modified epigallocatechin gallate-hydrophilic-lipophilic balance 6 in HeLa cells. Samples were added to the cells at concentrations of epigallocatechin gallate (EGCG) between 10 and 100 ppm and then incubated for 48 h. Each value represents a mean \pm SD of four replicates for surfactant-modified EGCG-HLB 4.3 and surfactant-modified EGCG-HLB 6 and three replicates for EGCG. HLB: Hydrophilic-lipophilic balance.

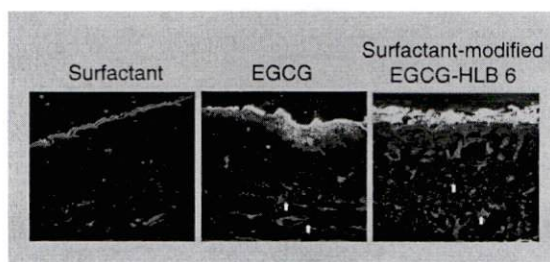
of EGCG. The results showed that surfactant-modified EGCG can produce higher cytotoxicity than native EGCG. The addition of Span 80 and Tween 80 increased the cytotoxicity of EGCG as shown in Figure 3. Surfactant-modified EGCG produced high cytotoxic effects with the IC₅₀ values of 90.15 and 58.71 ppm for HLB value of 4.3 and 6, respectively. On the other hand, EGCG demonstrated low cytotoxicity, as indicated by its IC₅₀ value >100 ppm. This study also evaluated the cell viability of control groups, which are DMSO and surfactants, and there were no remarkable cytotoxic effects on HeLa cells (data not shown). This is possibly due to the alteration of EGCG hydrophobicity, which became more lipophilic than native EGCG, thus increasing its ability to penetrate cell membrane. Koga *et al.* have reported that at nonsolubilizing concentrations Span and Tween altered membrane lipid fluidity related to hydrophobicity as reflected by the HLB number of these surfactants [29]. The lipid membrane changes were predominantly located in the outer leaflet of lipid membrane due to the interaction between fatty acid groups of surfactants and polar phospholipid head group of cell membrane. In addition, modifying the HLB system at values of 4.3 and 6 produced almost half of IC₅₀ although their respective relatively close log P values were 3 and 2.1. A previous report stated that a log P value of 2.5 becomes an intersection where, below this point, the penetrants will be favorably distributed within the dermis, while above it substances will more probably be partitioned into the epidermis [30], which is more lipophilic than a dermis stratum. The cell membrane is likely to possess similar characteristics to the dermis area, rather than the epidermis, due to its water and lipoprotein content.

It has been previously reported that cancerous cell are more sensitive to oxidative stress than normal cells and EGCG produce extracellular and intracellular oxidative stress, either through the production of hydrogen peroxide or semiquinone radicals [6]. A report also exists which argued that an increase in oxidative stress production occurs in cancer cell induced by EGCG while reducing reactive oxygen species levels in normal cells [31]. However, the cytotoxicity of surfactant-modified EGCG in normal cells was undetermined in this study. Further investigation into oxidative degradation stability of surfactant-modified EGCG and its cell cytotoxic sensitivity are required. The formation of reverse micelle improved cell permeability and entrapping EGCG inside may offer some protection for EGCG from oxidative degradation.

Penetration of EGCG into rat skin

It has been determined that log P indicates the lipophilicity of penetrants that plays a major role in skin permeation. Skin permeation of EGCG has been reported to be very low due to a high bound percentage (approaching 90%) of EGCG in skin tissue, thus reducing the degree and rate of skin permeation of EGCG during the diffusion

Figure 4. The photomicrographs of Rhodamine-labeled surfactant, surfactant-modified epigallocatechin gallate at an hydrophilic-lipophilic balance value of 6 and epigallocatechin gallate observed 2 h after topical application of samples into Wistar rat abdomens during an *in vivo* skin penetration study. The samples were observed through a fluorescence microscope at a magnification of 42 \times . The Rhodamine-labeled EGCG is indicated by a white arrow. EGCG: Epigallocatechin gallate.



process [15]. In this study, increasing the hydrophobicity of EGCG through modification of the log P value improved its penetration of the rat's skin. The skin penetration of EGCG and its modified forms were evaluated by observing the EGCG fluorescence of the skin tissue preparation after 2 h of application. This constitutes a prestudy to evaluate the permeation rate of surfactant-modified EGCG for topical vaginal routes. As shown in Figure 4, there was an increase in skin penetration for modified EGCG, which had been processed with surfactant addition, 2 h after application. We have also evaluated for skin penetration of these samples 5 h after application, and it resulted in no significant different from those of 2 h (data not shown). This modified EGCG demonstrated more intense fluorescence than EGCG solution. It was confirmed that surfactant addition did not affect observation of the fluorescence of the samples.

It is known that skin tissue consists of lipoprotein that has amphiphilic properties which probably cause the EGCG trapped in the reverse micelle core to more easily penetrate the skin layer than the native EGCG. In addition to this mechanism, the surfactants can act as enhancers that affect the integrity of the stratum corneum. It has been reported that the presence of surfactant, which has the function of an enhancer, also plays a role in the EGCG penetration process [20]. Therefore, it also improves the skin penetration of EGCG.

Conclusion

In summary, changes in the partition coefficient for improving the permeability of EGCG by micelle formation using a combination of Tween 80 and Span 80 were analyzed. The reverse micelle method successfully increased the lipophilicity of EGCG. The use of surfactant combination with a HLB value of 6 resulted in the log P value of EGCG being closest to that of skin, at 2–3, and producing higher cytotoxicity and more rapid and deeper skin penetration compared with native EGCG. Further evaluation of the anticancer activity of this modified EGCG is required to produce a high level of success of cervical cancer therapy.

Future perspective

Developing the topical delivery of EGCG by means of creams or in ovule-like dosage form represents an effective alternative means of supporting the treatment of cervical cancer. This study produced a positive approach to effectively increasing the EGCG permeability through skin by altering the log P value using a surfactant combination of Tween 80 and Span 80. As a preliminary investigation, this research represents a stepping stone in applying science to the development of therapeutic products for the treatment of cancer. Further research is definitely required to perfect and comprehensively define the delivery system. Evaluating drug tissue distribution would enable quantification of the effectiveness of the system in improving the skin penetration of EGCG. Moreover, further investigation of the surfactant-drug assemblies that may affect the morphological structures of EGCG-surfactant mixtures would play an important role in determining the detailed interaction between EGCG and surfactants, and the manner in which the system could improve the skin penetration of EGCG.

Financial & competing interests disclosure

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No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The animal experimental investigation used in this study was reviewed and complied with the guiding principles for the care and use of laboratory animals as set up by the formal ethics review committee on animal research of the Faculty of Veterinary Science, Universitas Airlangga, Indonesia.

Summary points

Topical delivery of epigallocatechin gallate for cervical cancer

- Epigallocatechin gallate (EGCG) has been reported to produce an apoptosis induction in cervical cancer.
- EGCG has high water solubility but has low permeability limiting topical delivery for therapeutic treatment of cervical cancer.

Log P value determining drug lipophilicity & skin penetration

- The optimal lipophilicity for topical drug penetration is indicated by the log P value within the range of 2–3.
- It has been reported that the use of surfactant could modify the log P value that influences physical stability, lipophilicity and skin penetration of a drug.

Combination of Tween 80 & Span 80 for improving permeability of EGCG

- Lowering the hydrophilic–lipophilic balance value by using a combination of Tween 80 and Span 80 increased the log P value of EGCG.
- Increasing the log P value of EGCG by addition of Tween 80 and Span 80 enhanced the cytotoxicity of EGCG.
- EGCG modified with Tween 80 and Span 80 produced improved penetration of a rat’s skin.

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Therapeutic delivery

37

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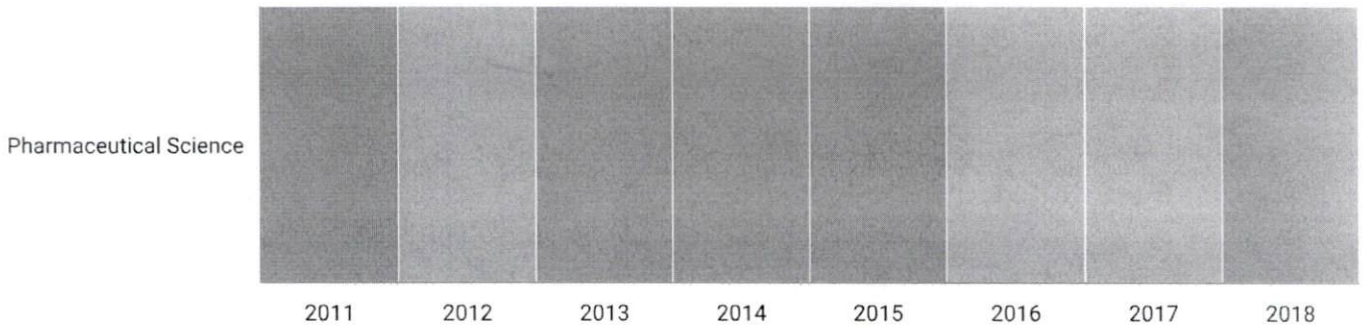
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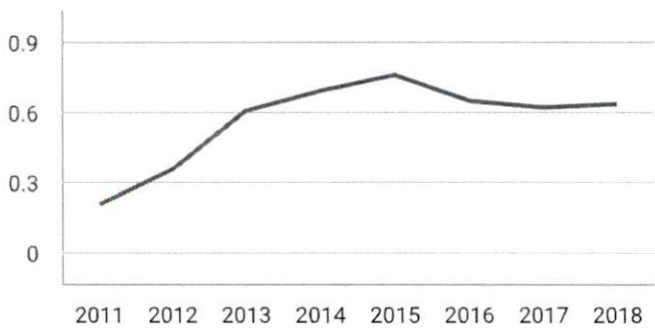
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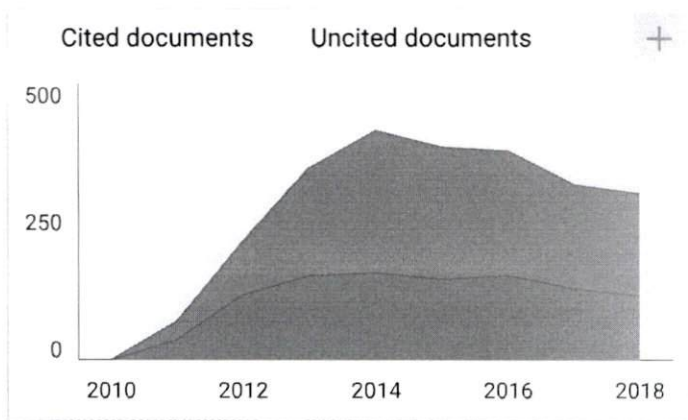
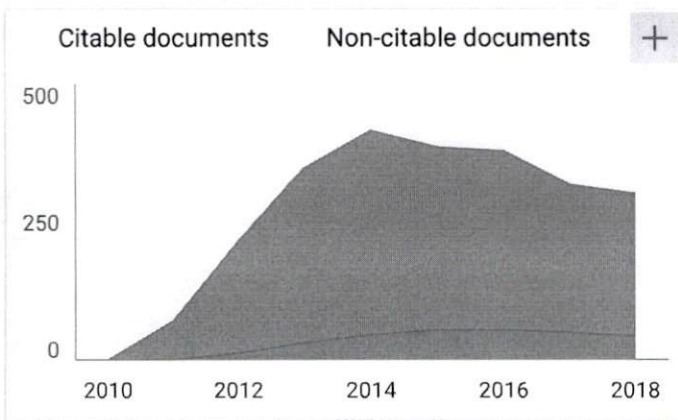
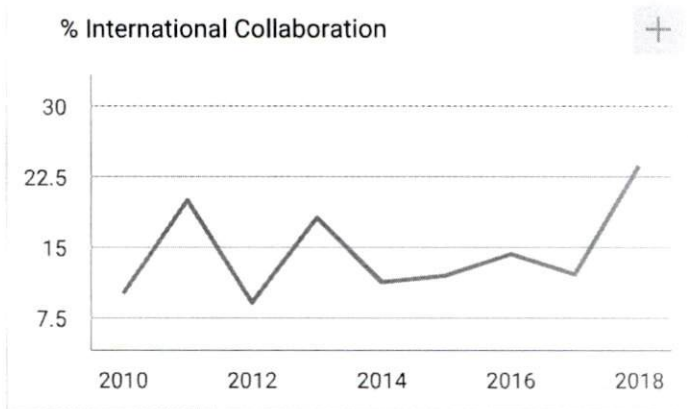
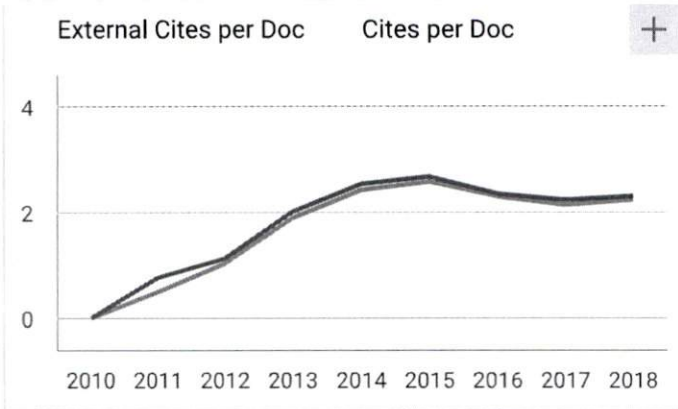
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