Journal of Advanced Biomedical and Pharmaceutical Sciences

Journal Homepage: http://jabps.journals.ekb.eg

Preparation and Evaluation of Ketotifen Suppositories

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Received: November 11, 2019; revised: November 29, 2019; accepted: December 5, 2019

Abstract

Ketotifen KT is one of antiallergic drugs, due to its first pass effect, the bioavailability of the drug is only 50 %. The objective of this study was to formulate and evaluate suppositories containing KT and/or KT solid dispersion.

The in-vitro release of KT from suppositories was done using dialysis membrane method in phosphate buffer at pH 7.4. The release of KT from water soluble suppository bases was higher than that from fatty or emulsion suppositories bases. Among all PEGs bases **(F4:** PEG 6000: PG (20: 80)**)** showed a relatively higher release of KT. Formulations prepared with glycerin bases gave more or less identical release pattern; relatively formula **(F17:** Gelatin: Glycerin: Propylene glycol: Water**)** gave the highest release pattern. Formula **(F20:** Suppocire AM**)** exhibited the highest release rate among fatty bases. Within all emulsion bases **(F23:** W_{15}: W_{75}: Tween 20: Span 60: PEG 1500: Propylene glycol**)** showed highest release rate. KT solid dispersion led to a higher release rate of the drug from selected bases.

A histological comparison between control group of rabbits (didn`t take suppository), another group took plain suppositories and group that received suppositories containing solid dispersion of KT was carried out. The tested plain and medicated bases didn't injure the rectal mucosa of rabbits. In conclusion the incorporation of solid dispersion in formula (**F4)** complied with the pharmacobeial limits for hardness, dissolution time, content uniformity and weight variation. Also it showed a relatively higher in-vitro release of KT and considered as safe and useful formulation for clinical use.

Key words

Suppository bases, Ketotifen, KT solid dispersion, In-vitro release, Histological studies

1. Introduction

Ketotifen (KT) is one of antiallergic agents which belong to the long term preventive medication of asthma as it is the second generation H_1-antihistamine drugs [1]

KT recommended dose is 2 mg/day divided into two doses [2]. KT is sparingly soluble in water 15.3 mg/L at 25 °C [3], which limits its dissolution prior to its absorption and hence could limit its bioavailability upon administration. In addition, KT is subjected to severe first pass effect and its bioavailability is only 50% after oral administration, also the drug is reported to be 75% protein bound [4]. KT is widely used as tablets, capsules, syrups, nasal drops and eye-drops (as fumarate salt) [2].

There is shortage of the availability of KT in the form of suppositories in literatures.

The advantages of rectal route over other routes of administration are due to the reduced side effects such as gastrointestinal irritation and the avoidance of both unpleasant taste and first pass effect. Furthermore, rectal route is suitable for children patients who cannot swallow medication and for patients with vomiting episodes [5, 6].

Consequently, rectal administration of KT in suppository form may exhibit apriority over its oral administration to enhance its bioavailability. Many studies have shown that the release characteristics of many suppositories depend on the physicochemical properties of the drug, suppository base and formulation additives [7-10] and a lot of formulations is normally required to optimize the maximum characters of suppository preparations.

For the preparation of proper suppository formulations, it is essential to select the ideal bases. An ideal base should be non-irritating to the sensitive tissues of the rectum. Unfortunately many suppository formulations, especially those prepared with the polyethylene glycol bases were reported to induce an irritation to mucous membranes [11]. Thus, the main objective of this study was to formulate and evaluate KT in a rectal dosage form, suppositories for children. Different formulations were prepared using water soluble PEG, gelatin bases, fatty and emulsion bases and investigated for their weight variation, drug content, hardness, disintegration time, melting range and *in-vitro* release. Furthermore, histological study on rabbit's rectal mucosa was performed to select the most safe and convenient suppository base.

2. Materials and Methods

2.1. Materials

Ketotifen was kindly supplied from (Pharco Co., Egypt.), Polyethylene glycol 600, 1500, 4000 and 6000 (Sigma Chem. Co., USA).Cocoa butter B.P. grade (Al-Goumhouria Co., Egypt). Witepsol H_{15}, Witepsol E_{75}, suppocire AM, suppocire CM (Gattefosse etablissements, France). Sodium alginate and sodium carboxymethyl cellulose (The General Chemical and

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Pharmaceutical Co., Ltd., England). Propylene glycol (Evans Chem. Co., Egypt).Tween 20 and Span 60 (Chemieliva Pharmaceutical Co., Ltd., China).Semi-permeable cellulose membrane, 12000-140000 MWCO (Sigma Chemicals, St. Louis, MO, USA). All other chemicals were of analytical grade and were used as received.

2.2. Preparation of KT solid dispersion

Solid dispersion of KT with hydroxypropyl-β-cyclodexetrin, (H-β-CD) at weight ratio 1:7 was prepared by the solvent evaporation method as follows. Weighed quantity of KT was dissolved in a minimum amount of absolute ethanol; the appropriate amount of (H-β-CD) was added. The resulting mixture was stirred until evaporation on magnetic stirrer and then the co-precipitates were then scrapped and stored in a desiccator over anhydrous $CaCl_2$, to constant weight. The evaporated product was ground in a mortar and passed through an 180μm sieve and stored in a desiccator until further evaluation. [12]

2.3. Preparation of KT suppositories

KT suppositories each containing 1mg of the drug and/ or KT solid dispersion with hydroxypropyl- β- cyclodextrin at ratio 1:7 (this ratio resulted in improving solubility as well as drug dissolution rate) [12] were prepared using different suppository bases (Table1-4). The fusion method was applied to formulate different suppository batches.

2.3.1. Preparation of KT water soluble and fatty suppositories bases

Firstly, the base was melted using water bath at suitable temperature then KT powder was added gradually to the melted base. Then, gentle stirring was continued to assure complete mixing and to enhance cooling. The mixture poured into a metal mold (1 g, standard suppository metal mold was made in Faculty of Engineering, Assiut University, Assiut, Egypt.) just before congealing. The metal mold was calibrated for displacement value of the drug. The selected water soluble suppository bases were blends of different molecular weight polyethylene glycol and another set of glycero-gelatin suppository bases. The fatty bases used were cocoa butter, suppocire CM, suppocire AM and witepsol H_{15}.

2.3.2. Preparation of KT emulsion suppositories bases

The emulsion bases consist of witepsol H_{15}, witepsol E_{75} or cocoa butter as the oily phase, while water and PEGs were used as the aqueous phase. Certain surfactants as tween 20 and span 60 were used as emulsifying agents. Suppositories of emulsion bases were formulated by solubilizing the surfactant in the hydrophilic or lipophilic phase and the polymer was solubilized in water phase. The used bases were melted then the aqueous phase in which the drug is dissolved was added with continuous agitation [13]. The mixture was finally poured into a metal mold.

After solidification at room temperature the formulated suppositories were packed in tightly closed containers and kept in a refrigerator. The suppositories were left for two hours at room temperature before use.

As KT is hydrophobic, the selection of fatty bases was just used to predict the release pattern of the drug from these lipophilic bases.

2.4. Evaluation of plain and medicated suppositories

2.4.1. Weight Variation

The weight variation test was estimated according to the British Pharmacopoeia 2007 [14]. Briefly, twenty suppositories were weighed individually and the average weight was calculated. No suppositories should deviate from average weight by more than 5% except two, which may deviate by not more than 7.5%.

2.4.2. Disintegration time

The test was completed in distilled water at 37°C using the U.S.P tablets disintegration apparatus (Erweka DT-D6, Heusenstamm, Germany). The disintegration time was registered as soon as the suppositories placed in the basket either totally melted or dissolved [15].

2.4.3. Hardness (Fracture point) Determination [16, 17]

Measuring the brittleness and fragility of the suppositories, a hardness teste was adopted. Hardness was determined at room temperature using a hardness tester (Erweka hardness tester, SBT, Heusenstamm, German.) The weight in Kg required for the deformation and breaking of the suppositories was determined.

2.4.4. Melting range determination

The test was executed using the capillary method [18] in electro thermal melting point apparatus (Gallenkamp, England). A standard capillary tube of 8 to 10 cm in length and 1 to 1.2 mm in diameter, opened at both ends was used. One end of the tube was immersed into the suppository bases and sufficient amount was packed to fill about 1 cm column. The capillary tube was then placed in the apparatus attached to a thermometer. The melting range was recorded when the contents of the capillary tube started to melt.

2.4.5. Uniformity of drug content

The British Pharmacopeia (2007) [14] method was applied. Ten suppositories were randomly chosen from each formula and individually assayed for drug content. A pre weight suppository dispersed in 25 ml of phosphate buffer pH 7.4 and allowed for gentle heating to melt in a water bath (Gallenkamp, Loughborough, UK) and then the volume was completed to 100 ml by the same buffer. The containers were allowed to agitate in water bath for two hours at maintained temperature 37±0.5°C. Samples were withdrawn, filtered using 0.45 μm membrane filter (Gelman Instrument Co.), suitably diluted and assayed spectrophotometrically (Jenway UV single beam spectrophotometer Feslted, Dunmow, U.K.,) at λ_{max} 301nm [19]

against a blank solution prepared by handling plain suppositories by the same procedure.

2.4.6. In-vitro drug release in phosphate buffer pH 7.4 by dialysis method [20]

Cellophane membrane previously soaked in phosphate buffer pH 7.4 was firmly stretched over the end of a glass tube (about 20 mm internal diameter and 15 cm in length). The tube was suspended in a 100 ml glass beaker containing 50 ml of phosphate buffer pH 7.4. A volume of 10 ml phosphate buffer was poured into the glass tube. The system was placed into a constant temperature shaker water bath (37±0.5°C, 100 rpm) and one suppository was introduced into the tube. Samples, each of 5 mL, were withdrawn from the release medium at specified time intervals and replaced by fresh buffer. The samples were filtered through 0.45 µm membrane filter and analyzed spectrophotometrically at λ_{max} 301nm [19] against a blank of plain suppositories treated by the same procedure for medicated suppository. Sink conditions are maintained during the whole experiment. The results are reported as the mean values of three release experiments. The cumulative percent drug released was plotted against time.

2.4.7. Kinetics of the KT release from suppositories bases

In order to determine the drug release mechanism, the in-vitro release data were fitted into different kinetic models of zero-order, first order and Higuchi diffusion models.

Zero-order release $m_0 - m = Kt$ **(1)**

First-order release $\log m = \log m_0 - Kt/2.303$ **(2)**

Higuchi model $m_0 - m = Kt1/2$ **(3)**

Where m is the amount of the drug remaining in the formulation at time t and m_0 is the initial amount of the drug in the formulation [21-23]. The correlation coefficient values (R) were calculated for all the models.

2.5. Administration of suppositories and investigation of the rectal mucosal changes

Experiments were carried out according to the animal ethics guidelines of Assiut University, Egypt. Selected plain and medicated suppositories of rabbit size containing KT solid dispersion (SD.) equivalent to (1mg) of KT were prepared by fusion method. The selected formulations are:

1- (F4) which contained PEG 6000: PG (20:80 %w/w).
2- (F17) which contained Gelatin: Glycerin: Propylene glycol: Water (14:6:40:20%w/w).
3- (F20) which contained 100% Suppocire AM.
4- (F23) which contained Witepsol H_{15}: Witepsol E_{75}: Tween 20: Span60: PEG 1500: Propylene glycol (20:10:20:5:1:40:20 %w/w).

Healthy New Zealand rabbits of either sex weighing about 1.5-2.0Kg (Animal house of faculty of Medicine, Assiut University-Egypt) were used. The rabbits were kept under control for one week before study and were kept on standard pellet-diet and tap water and were housed at room temperature [24].

For each study, one suppository was inserted deeply into the rectum of the rabbit. The anus was closed immediately after insertion with a thick plaster for one hour to prevent any leakage. The insertion was repeated daily for ten days. At the end of this period the rabbit was sacrificed. The rectum including the anus was removed as one segment (about 5cm in length) and preparations of rectal segments for microscopic observations were made. The preparations were stained with haematoxylin and eosin and examined by the light micro-scope. Three rabbits were used for each formula as well as for the control (untreated rabbits).

2.6. Statistical analysis

All experiments were carried out in three independent experiments, and the results were recorded as mean ± standard deviation (SD). Statistical analysis of the release data of KT from selected suppositories base F4, F17, F20 and F23 and their corresponding formulae containing KT/ H-β-CD (1:7) SD. was implemented utilizing one-way ANOVA test by means of (Graph pad prism program, version 5, San Diego, USA). All statistically significant differences were anticipated when $p<0.05$.

3. Results and Discussion

3.1. Variation of weight, disintegration time, hardness, melting range determination and drug content uniformity

The formulated suppositories were totally formed with fine and shinny surface, white or whitish in color for PEGs, fatty and emulsion bases and appeared yellow in case of gelatin base. The suppositories did not show any fissures, cracks or holes when longitudinally cutting.

It was found that, the weight variation for all tested suppositories within the acceptable range of < 5 % **(Table 1-4)**, that indicated ideal standardization of mold.

(Table 1-4) show dissolution times of different suppository formulations. They are either dissolved or softened and melted within the range of 13-26 min, 18-23 min, 3-6 min, 5-24 min for polyethylene glycol, gelatin, fatty and emulsion, respectively. The melting time, for a fat based suppositories should not exceed 30 minutes, while dissolution time for water soluble suppositories should not exceed 60 minutes as declared by The B.P. (2007) [15].

The mechanical strength for the formulated suppositories was in the range of 1 to4.6 kg demonstrating optimum hardness for handling, shipping and insertion. **(Table 1-4)**

The tested formulations showed remarkable variability in melting point determination. A narrow melting range is significant in preserving the shape of the suppository in room temperature and in controlling the melting time of the suppository after insertion. Witepsol H_{15} (F21) has the lowest melting range among the other fatty bases **(Table 3)**. Within emulsion bases, Witepsol based suppositories (F23, 24) showed

higher melting range than cocoa butter based one (F22) as demonstrated in **table 4.**

Drug content was established to comply with the demands of B.P. (2007) [14], the range was from 98.48 – 101.67 % of the incorporated amount. **(Table 1-4)**

All the previous results showed no differences between the physical characteristics of plain and medicated suppositories.

3.2. In-vitro release of KT from different suppository formulation into phosphate buffer at pH 7.4

As there is no standard official recognized technique or apparatus system designed for the release study of drugs from suppositories, many researches have been carried out.

Both direct contact and dialysis methods have been utilized with various modifications [25]. Methods of suppository dissolution testing without membrane have been developed by simple adjustment of the USP tablet dissolution apparatus [26-30].

In this study, the dialysis technique was used because the drug release will be in a condition similar to that of the rectum [31-33].

3.2.1. In-vitro release of KT from water-soluble polyethylene glycol (PEGs) bases

The in-vitro release of KT from water-soluble bases (F1-F13) is demonstrated in **(Table 1)** and **(Figures 1-4).** It is clear that the release can be affected by the presence of propylene glycol and liquid PEGs (600 and1500) and the percent of solid PEGs (6000 and 4000) in the suppository base which contributed to enhance solubility and dissolution in the aqueous medium.

Increasing concentration of solid PEGs (6000 and 4000) at the same time with decreasing the concentration used from PEG 600, PEG1500 or propylene glycol resulted in rising the melting point and increasing hardness of the base as well as the hydration process by water uptake followed by formation of gelatinous layers which leading to retardation in the in-vitro release of the drug and vice versa.

According to the obtained results, (F4) which contained 20 % PEG 6000 and 80 % PG showed the highest release of the drug among the other water soluble polyethylene glycols suppository bases used (F1-F13). These results are in a good harmony with the previously reported results that showed a higher release of propranolol hydrochloride, fenbufen, mebeverine, lornoxicam and diclofenac sodium from hydrophilic bases compared to lipophilic bases [13, 31, 34-36].

3.2.2. In-vitro release of KT from water-soluble gelatin bases

(Table 2) and **(Figure 5)** represent KT release from different gelatin bases. The tested bases followed the following rank: **F17>F16>F15>F14**

The percentage of KT released after 120 minutes from F17, F16, F15 and F14 were 54.99 %, 47.907 %, 39.667 % and 30.066 % respectively. These results could be clarified on the basis that by increasing propylene glycol concentration lead to a decrease in the dissolution time and as well as it has an enhancing effect on the drug solubility [28].

Due to the hypertonic property of glycerin, it has been reported that in gelatin suppository formulations propylene glycol and polyethylene glycol 600 have been utilized as complete or partial substitutes for glycerin which is also not as good solvent as the substitute materials [37].

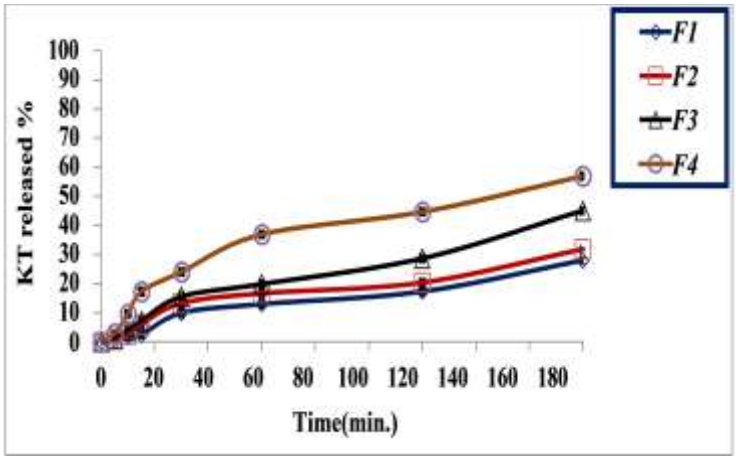


Figure 1: In-vitro release of KT from different water-soluble PEGs suppository bases (F1-F4) into phosphate buffer at pH 7.4 and 37 ºC.

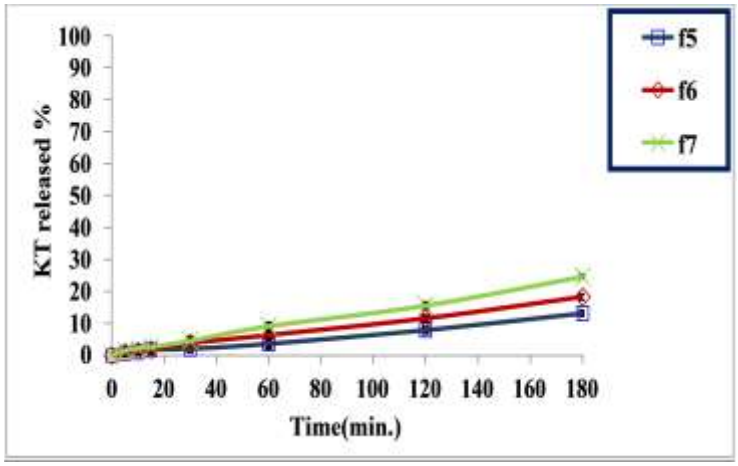


Figure 2: In-vitro release of KT from different water-soluble PEGs suppository bases (F5-F7) into phosphate buffer at pH 7.4 and 37 ºC.

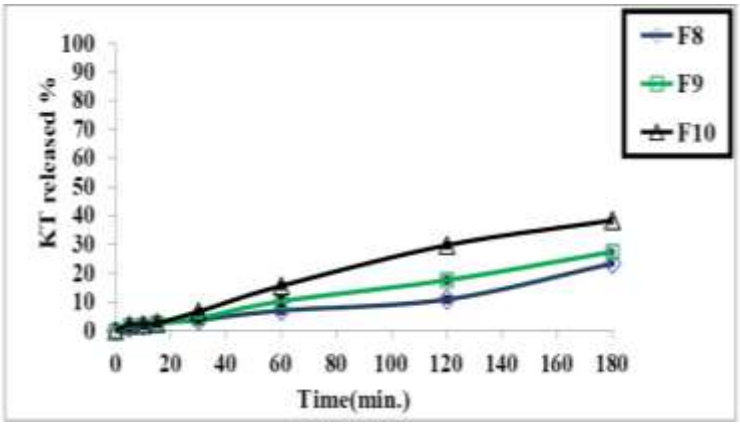


Figure 3: In–vitro release of KT from different water-soluble PEGs suppository bases (F8-F10) into phosphate buffer at pH 7.4 and 37 ºC.

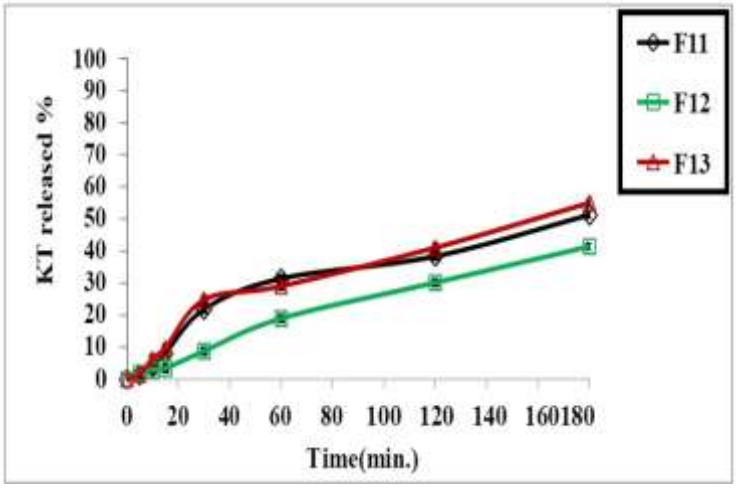


Figure 4: In-vitro release of KT from different water-soluble PEGs suppository bases (F11-F13) into phosphate buffer at pH 7.4 and 37 ºC.

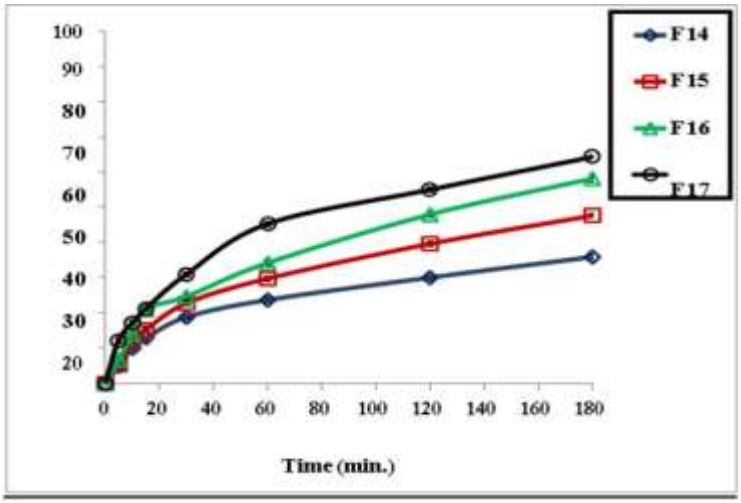


Figure 5: In vitro release of KT from different water-soluble gelatin suppository bases (F14-F17) into phosphate buffer at pH 7.4 and 37 ºC.

3.2.3. In-vitro release of KT from fatty bases

The KT release results from these bases were less than that obtained from the water-soluble and emulsion bases and this was predictable due to the more affinity of the hydrophobic KT to the lipophilic bases as shown in the **(Table 3**) and **(Figure 6)**. The tested bases followed the rank of: **F20>F21 >F19 >F18 Suppocire AM >Witepsol H15 >Suppocire CM > Cocoa butter**

These results can be assigned to dependency of the release on melting behavior, chemical composition of base and the partition coefficient of KT between the base and the buffer.

Synthetic suppository bases are mixtures of fatty acid esters with certain amounts of glycerides. Their hydroxyl values represent the presence of mono and diglycerides, which also indicate the availability of free hydroxyl groups in the bases [37]. It was reported that higher release of many drugs was expected from bases with low hydroxyl values which reflects the low affinity of the drugs to these bases [38]. In the present formulations, suppocire AM (F20) gave higher release than witepsol H_{15} (F21), this may be attributed to the lower hydroxyl value of suppocire AM (≤ 6) in comparison to witepsol H_{15} (≤ 15) [39], in addition to the short dissolution time of suppocire AM as shown in **(Table 3)**.

Calis et al. [38] reported that the release of the potent antimicrobial agent (C31G) was higher from suppocire than from witepsol H_{15}.

Although, Witepsol H_{15} (F21) has melting range (34-35ºC) as same as that of cocoa butter (F18), it gave higher KT release. This is can be explained by the difference in chemical composition between them. The presence of self emulsifying agents in witepsol H_{15} may facilitate the dispersion of KT in the surrounding medium [40].

It is clear that cocoa butter showed the slowest release among the other emulsion bases. This is due to the existence of monoglycerides esters in both suppocire and witepsol H_{15} bases which work as self emulsifiers resulting in high emulsifying and water absorbing capacities accountable for increasing drug release [13].

A good agreement between these results and the reported results of higher release of ciprofloxacin hydrochloride and propranolol hydrochloride from witepsol H_{15} based suppositories than from cocoa butter suppositories.

In the case of suppocires, the KT release from suppocire AM (F20) was found to be higher than suppocire CM (F19). This is may be due to the high melting range of suppocire CM (38-39ºC) compared to that of suppocire AM (35-36.5ºC) [17, 38].

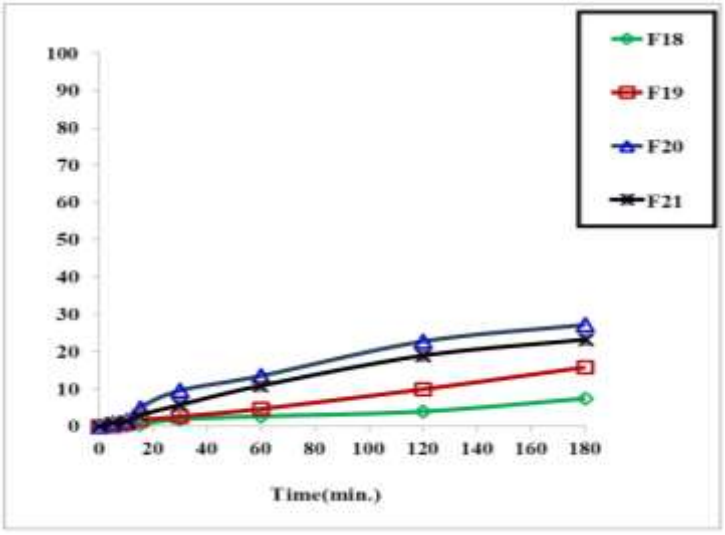


Figure 6: In-vitro release of KT from different fatty suppository bases (F18-F21) into phosphate buffer at pH 7.4 and37 ºC.

3.2.4. In-vitro release of KT from emulsion bases

The in vitro release of KT from emulsion suppositories is listed in **(Table 4)** and demonstrated in **(Figure 7**). The emulsion bases followed the rank of: **F23>F22 >F24**

The melting range and the dissolution time of the suppository base are dependent on the base components. In this regard, witepsol H_{15} when used as the oily phase led to the formation of an emulsion with low melting range and short dissolution time compared to witepsol E_{75}. Presence of PEG 1500 with propylene glycol in the base as the aqueous phase instead of water resulted in a higher KT release rate. This might be due to

the concomitant rapid dissolution of the suppository and the influence of PG and PEGs on the solubility of drug.

The relative enhancement of KT release from emulsion bases may be due to the presence of nonionic surfactant, tween 20, which improves the wettability of the base and increases the dispersion of the drug into the surrounding medium [31,39]. Also, the surfactant may increase the rate of diffusion through the cellophane membrane [29].

Suppositories contained sodium CMC (F24) displayed small lower release in comparison to suppositories containing sodium alginate (F22), this could be due to the higher gelling effect offered by sodium CMC [42].

From the results of the release of KT from different suppository bases can be ranked follows: **Water soluble bases > Emulsion bases > fatty bases**

A good agreement of these results with those obtained by El-Nabarawi et al., [43] who worked on tramadol hydrochloride suppositories and reported that drug released more rapidly from hydrophilic bases than lipophilic ones and the release of isoconazole nitrate [44] was higher from hydrophilic bases compared to lipophilic ones.

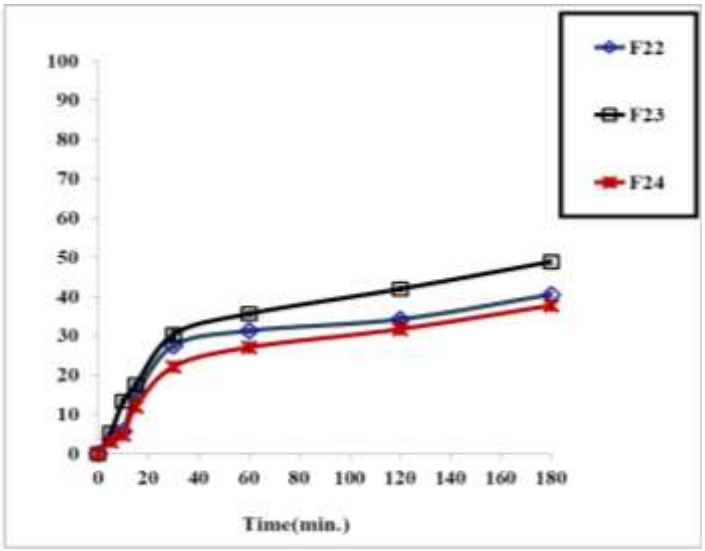


Figure7: In-vitro release of KT from different emulsion suppository bases (F22-F24) into phosphate buffer at pH 7.4 and 37 ºC.

3.3. In-vitro release of KT from selected suppository bases containing KT solid dispersion with H-β-CD (1:7)

(Figures 8-12) show the release profiles of KT from the selected suppository bases (water soluble PEG base, F4), (water-soluble gelatin base, F17), (fatty base, F20) and (emulsion base, F23) respectively containing solid dispersion of KT/ H-β-CD (1:7), into phosphate buffer at pH 7.4.

The selected suppository formulae were utilized to study the effect of solid dispersion incorporation on their physical parameters as shown in **tables 1-4**. The results revealed that solid dispersion has no effect on the physical characteristics of the tested bases.

The KT solid dispersion (SD. KT) led to a higher release rate of the drug from each of the selected bases compared to that of the untreated drug. The improved drug release after the incorporation of solid dispersion may be due to the increased wettability, solubilization, and transformation of the drug from the crystalline state to the amorphous one [45]. This was confirmed by DSC, P-XRD and SEM through the formation of drug-solid dispersion with H-β-CD in our previous study [12].

It was reported that solid dispersions of azapropazone with PVP K30 and surface deposition of azapropazone with fluorite were used in preparing suppository formulations using hydrophilic suppository base (mixtures of PEGs). The drug release rate from the base was remarkably increased using solid dispersion and solvent deposition techniques in comparison to the untreated drug [46].

Statistical analysis of the release data of KT from F4, F17, F20 and F23 in addition to the corresponding formulae containing KT/ H-β-CD (1:7) SD. was done by ANOVA test. It was found that there was a **highly significant difference** between the release from suppository formulae containing only the drug (F4, F17, F20 and F23) and that containing KT SD. ($P \leq 0.001$).

Also it was found that there was a **significant difference** between the amount release of KT from formula (F4 SD.) and that obtained from formula (F17 SD.) ($P \leq 0.01$) till the first 30 minutes, then a **non significant difference** between them till the end of the 180 minutes ($P > 0.05$).

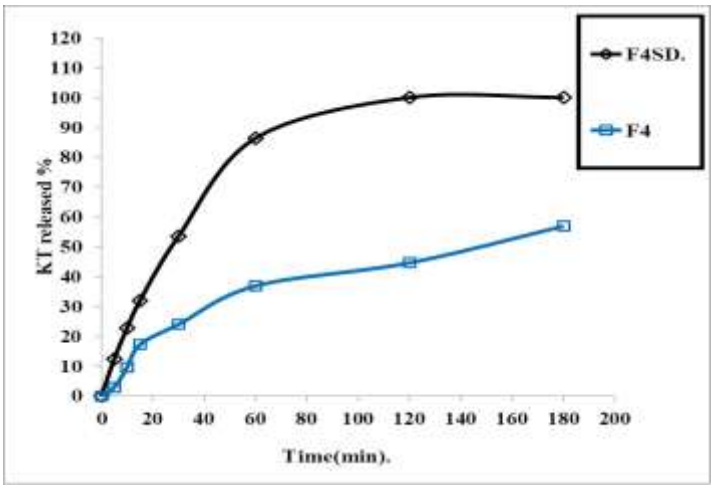


Figure 8: In-vitro release of KT from the selected base F4 containing SD. of KT with HP-β-CD (1:7) in phosphate buffer pH 7.4 and 37ºC.

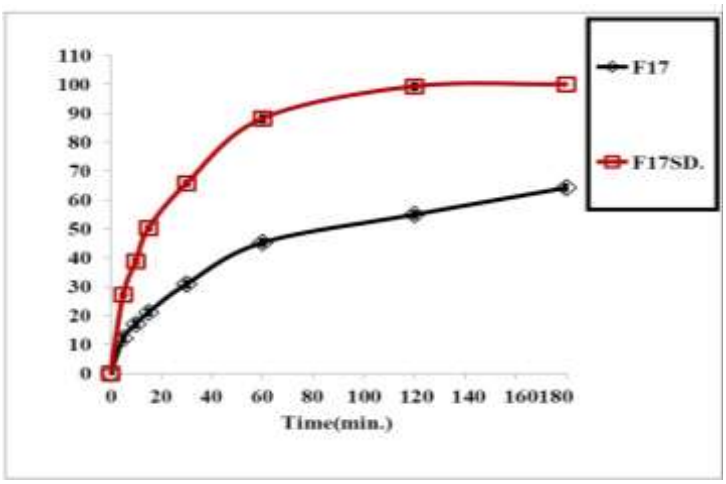


Figure 9: In-vitro release of KT from the selected base F17containing SD. of KT with H-β-CD (1:7) in phosphate buffer pH 7.4 and 37ºC.

Table (1): Composition and characterization of water soluble PEG suppository formulations.

	Suppository composition	Average weight (g ± S.D)*			Dissolution Time (min.± S.D) #			Hardness (kg ± S.D)#			Melting range (° C) #			Drug content in medicated suppositories (mg ± S.D)**		Amount released at 180 min (%)	
		Plain	Medicated	Solid dispersion	Plain	Medicated	Solid dispersion	Plain	Medicated	Solid dispersion	Plain	Medicated	Solid dispersion	Medicated	Solid dispersion	Medicated	Solid dispersion
F1	PEG 6000: PG 60:40	1.096 ± 0.022	1.168 ± 0.007		20 ± 1.16	22 ±0.52		1.6 ± 0.15	1.8 ± 0.33		-	-		98.99 ± 1.01		27.973 ±0.681	
F2	PEG 6000: PG 50:50	1.11 ± 0.026	1.193 ± 0.014		18 ± 0.45	19 ±0.37		1.4 ± 0.44	1.6 ± 0.21		-	-		99.93 ± 0.91		31.847 ±0.266	
F3	PEG 6000: PG 40:60	1.118 ± 0.009	1.131 ± 0.02		17 ± 0.76	18 ±0.68		1 ± 0.43	1.20 ± 0.51		-	-		100.12 ± 0.88	101.87 ±0.56	45.036 ±0.835	
F4	PEG 6000: PG 20:80	0.989 ± 0.017	0.966 ± 0.029	1.023 ±0.044	14 ± 1.03	13 ±0.92	14± 1.89	1.2 ± 0.35	1.2 ± 0.62	1.4 ± 0.83	38-39	39-40	39-40	100.97 ±0.34		**56.977 ±0.911**	**99.80± 0.921**
F5	PEG 6000: PEG 600 60:40	1.123 ± 0.011	1.29 ± 0.03		24 ± 1	26 ±0.27		4.4 ± 0.78	4.4 ± 0.4		-	-		100.52 ±0.59		13.153 ±0.691	
F6	PEG 6000: PEG 600 50:50	1.006 ± 0.034	1.086 ± 0.047		23 ± 0.2	23 ±0.49		4 ± 0.29	4.2 ± 0.57		-	-		99.16± 0.37		18.257 ±0.898	
F7	PEG 6000: PEG 600 40:60	1.202 ± 0.006	1.11 ± 0.024		21 ± 0.26	23 ±0.41		3.8 ± 0.47	4 ± 0.31		-	-		100.02 ±0.87		24.617 ±0.305	
F8	PEG 4000: PEG 600 60: 40	0.981 ± 0.062	1.009 ± 0.087		22 ± 0.53	24 ±0.61		3.4 ± 0.57	3.2 ± 0.28		-	-		101.37 ±0.38		23.417 ±0.428	
F9	PEG 4000: PEG 600 50:50	1.106 ± 0.033	1.2 ± 0.06		20 ± 0.35	21 ±0.83		3 ± 0.39	3.2 ± 0.19		-	-		99.28± 0.73		27.443 ±0.480	
F10	PEG 4000: PEG 600 40:60	0.989 ± 0.071	1.009 ± 0.012		19 ± 1.11	22 ±0.89		2.6 ± 0.28	2.6 ±0.4		-	-		100.15 ±0.19		38.38± 0.554	
F11	PEG 6000: PEG 1500 30:70	0.940 ± 0.087	0.966 ± 0.014		22 ± 0.66	22 ±0.87		4 ± 0.42	4.2 ± 0.27		-	-		99.18± 0.54		51.187 ±0.301	
F12	PEG 6000: PEG 1500 : water 50:30:20	0.906 ±0.033	0.972 ± 0.018		23 ± 1.04	24 ±0.91		4.4 ± 0.37	4.6 ± 0.13		-	-		98.48± 0.78		41.37± 0.459	
F13	PEG 4000: PEG 1500 25:75	0.991 ± 0.083	1.006 ± 0.009		20 ± 0.58	21 ±0.84		3.8 ± 0.11	4 ± 0.19		-	-		99.97± 0.61		54.977 ±0.761	

Results represent mean ± standard deviation of **5** observations.
***** Results represent mean ± standard deviation of **20** observations.
****** Results represent mean ± standard deviation of **10** observations.

Table (2): Composition and characterization of water soluble gelatin suppository formulations.

Code	Suppository composition	Average weight (g ± S.D)*			Dissolution Time (min.± S.D) #			Hardness (kg ± S.D)#			Melting range (° C) #			Drug content in medicated suppositories (mg ± S.D)**		Amount released at 180 min (%)	
		Plain	Medicated	Solid dispersion	Plain	Medicated	Solid dispersion	Plain	Medicated	Solid dispersion	Plain	Medicated	Solid dispersion	Medicated	Solid dispersion	Medicated	Solid dispersion
F14	Gelatin: Glycerin: Water 14:46:40	0.948 ± 0.065	0.997 ± 0.018		22 ± 0.84	23 ±1.22		-	-		-	-		98.55± 0.83		35.923 ±0.751	
F15	Gelatin: Glycerin: Propylene glycol: Water 14:26:20:40	0.979 ± 0.078	1.17 ± 0.009		20 ±1	21 ±0.78		-	-		-	-		99.87± 0.65		47.683 ±0.485	
F16	Gelatin: Glycerin: Propylene glycol: Water 14:16:30:40	1.103 ± 0.02	0.998 ± 0.053		19 ± 1.12	22 ±0.32		-	-		-	-		100.24± 0.34		58.11± 1.112	
F17	Gelatin: Glycerin: Propylene glycol: Water 14:6:40:40	1.021 ± 0.04	1.098 ± 0.01	1.044 ±0.69	18 ± 0.21	18 ±0.69	17± 0.58	-	-	-	-	-	-	101.02± 0.11	99.08 ±0.138	**64.273 ±0.146**	**98.01± 0.136**

Results represent mean ± standard deviation of **5** observations.
***** Results represent mean ± standard deviation of **20** observations.
****** Results represent mean ± standard deviation of **10** observations.

Table (3): Composition and characterization of fatty suppository formulations.

Code	Suppository composition	Average weight (g± S.D)*			Dissolution Time (min.± S.D) #			Hardness (kg ± S.D)#			Melting range (° C) #			Drug content in medicated suppositories (mg ± S.D)**		Amount released at 180 min (%)	
		Plain	Medicated	Solid dispersion	Plain	Medicated	Solid dispersion	Plain	Medicated	Solid dispersion	Plain	Medicated	Solid dispersion	Medicated	Solid dispersion	Medicated	Solid dispersion
F18	Cocoa butter	0.992± 0.062	1.004 ± 0.034		3 ± 1	5 ± 0.2		1.6 ±0.56	1.8 ± 0.12		34-35	34-36		100.22 ±0.5		7.3866 ±0.428	
F19	Suppocire CM	0.953± 0.009	0.899 ± 0.085		5 ± 0.51	6 ± 0.78		1.4 ±0.24	1.6 ± 0.31		37-38	38-39		100.91 ±0.63		15.77± 0.875	
F20	Suppocire AM	0.987 ± 0.017	0.991 ±0.051	0.995 ±0.077	4 ± 0.79	5 ± 1.01	5± 1.12	1± 0.43	1.2 ± 0.7	1.2 ± 0.92	36-37	36-37	37-38	99.74 ± 1.07	100.54 ± 0.92	**27.187 ±1.211**	**48.51 ± 1.110**
F21	Witepsol H15	0.956± 0.078	0.983 ± 0.006		5 ± 1.12	6 ± 0.45		1 ± 0.29	1.2 ± 0.52		33-34	34-36		100.99 ± 0.21		23.177 ±0.676	

Results represent mean ± standard deviation of **5** observations.
***** Results represent mean ± standard deviation of **20** observations.
****** Results represent mean ± standard deviation of **10** observations.

Table (4): Composition and characterization of emulsion suppository formulations.

Code	Suppository composition	Average weight (g ± S.D)*			Dissolution Time (min.± S.D) #			Hardness (kg ± S.D)#			Melting range (° C) #			Drug content in medicated suppositories (mg ± S.D)**		Amount released at 180 min (%)	
		Plain	Medicated	Solid dispersion	Plain	Medicated	Solid dispersion	Plain	Medicated	Solid dispersion	Plain	Medicated	Solid dispersion	Medicated	Solid dispersion	Medicated	Solid dispersion
F22	Cocoa butter: Sodium alginate: Tween 20: Distilled water 74:2:4:20	0.889± 0.02	0.991 ± 0.032		5 ± 0.92	7 ±0.27		1.4 ± 0.61	1.6 ± 0.13		33-34	34-35		101.67 ±0.46		40.48± 0.7033	
F23	W15: W75: Tween 20: Span 60 : PEG 1500: Propylene glycol 24: 10: 5: 1: 40: 20	0.875± 0.033	0.868 ± 0.051	0.993 ± 0.046	19 ± 1.03	19 ± 0.78	20 ±0.32	1.2 ± 0.34	1.4 ± 0.55	1.4 ± 0.76	35-36	35-36	36-37	100.03 ± 0.29	99.98 ± 0.41	**48.836 ± 0.605**	**60.01 ± 0.555**
F24	W75: Sodium CMC: Tween 20 : Distilled water 50: 1: 4: 45	0.994± 0.067	1.001 ± 0.014		24 ± 0.56	23 ±1		1 ± 0.19	1 ± 0.23		36-37	37-38		99.65 ± 0.88		37.68 ± 0.363	

Results represent mean ± standard deviation of **5** observations.
***** Results represent mean ± standard deviation of **20** observations.
****** Results represent mean ± standard deviation of **10** observations.

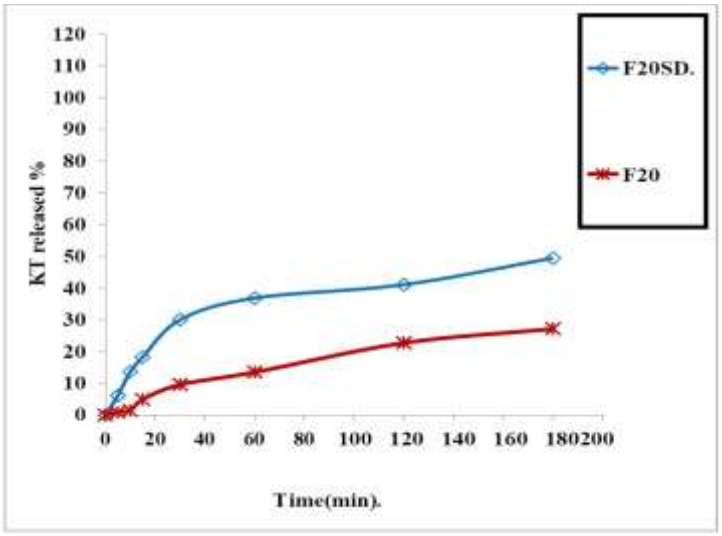


Figure 10: In-vitro release of KT from the selected base F20 containing SD. of KT with HP-β-CD (1:7) in phosphate buffer pH 7.4 and 37ºC.

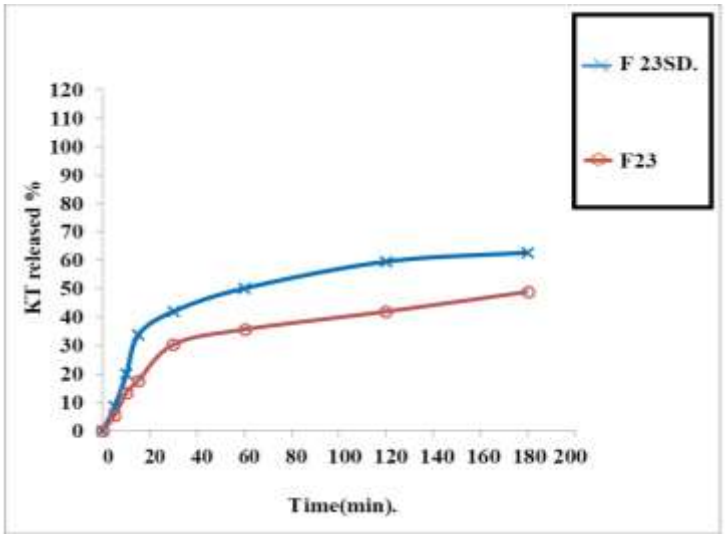


Figure 11: In-vitro release of KT from the selected base F23 containing SD. of KT with HP-β-CD (1:7) in phosphate buffer pH 7.4 and 37ºC.

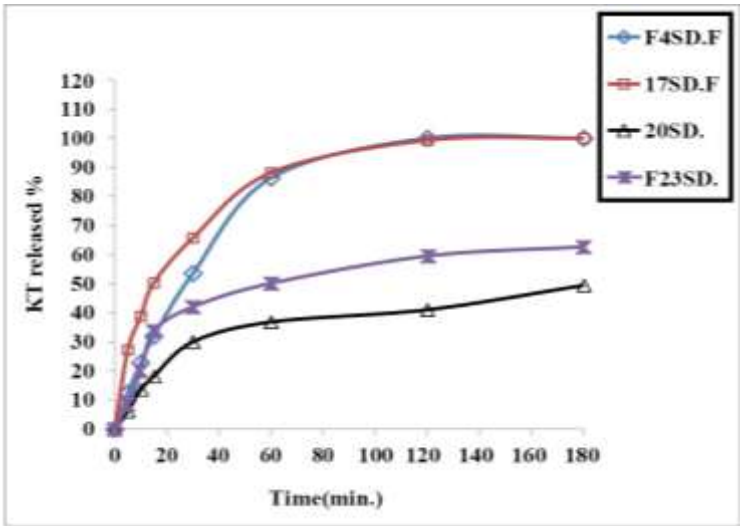


Figure 12: In-vitro release of KT from the selected base F4, F17, F20 and F23 containing SD. of KT with HP-β-CD (1:7) in phosphate buffer pH 7.4 and 37ºC.

3.4. Kinetic analysis of the release of untreated KT and KT solid dispersion from the prepared suppository bases

In order to get insights into the mechanism of drug release, linear regression analysis of the data of KT release from the water-soluble PEGs bases (F1-F13), water-soluble gelatin bases (F14-F17), fatty bases (F18-F21) and emulsion bases (F22-F24) as well as the release of KT solid dispersion with hydroxypropyl-β-cyclodexetrin (1:7) from selected suppositories bases into phosphate buffer at pH 7.4 was fitted into zero, first order kinetics and Higuchi model of diffusion equations [22]. Results are summarized in **(Table 5, 6).**

In case of similarity in coefficient of variation between zero-order and Higuchi model of diffusion Schwartz slope was used to differentiate between them. Deviation of Schwartz slope from 0.5 declines the Higuchi model of diffusion [23].

The in vitro release results showed that the release of KT from different suppositories bases is most fitted to zero order mechanism according to the higher correlation coefficient. These results are as same as the reported result obtained from Muaadh A M Ali [47] who found that, the release pattern of metoclopramide Hcl from suppositories was fitted to zero order mechanism.

3.5. Histological studies of the effect of selected suppository formulations on the rectal mucosa of rabbits

For the preparation of proper suppository formulations, it is essential to select the ideal bases. An ideal base should be non-irritating to the sensitive tissues of the rectum. Unfortunately many suppository formulations, especially those prepared with the polyethylene glycol bases were reported to induce an irritation to mucous membranes [11].

Photomicrographs of the rabbit rectal mucosa after chronic treatment with 8 different samples for 10 days are illustrated in **(Figures 13-17).**

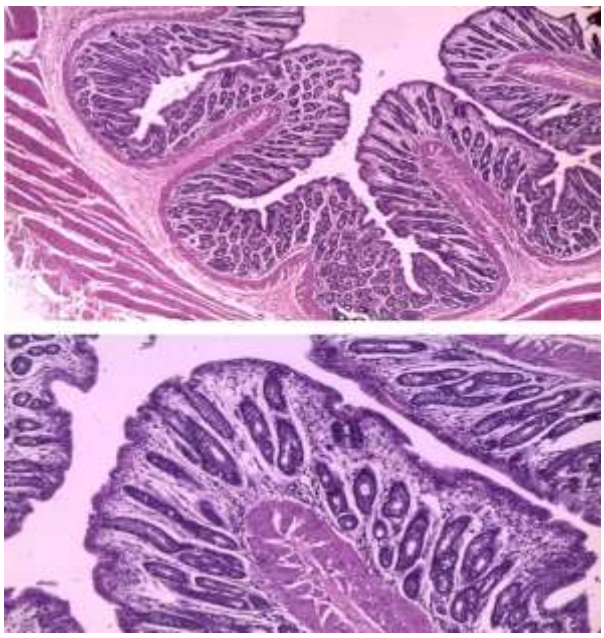


Figure 13: Shows the normal rectal mucosa of the control group of rabbits.

Table (5): Release characteristics of KT from water soluble suppository formulations.

Code	Suppository composition	Zero Order R^2	First order R^2	Higuchi Diffusion model R^2	K Value (mg ml^{-1} min^{-1})	Order of release	Order of release After Schwartz slope was deviated from 0.5.
F1	PEG 6000: PG 60:40	0.9777	-0.5402	0.9644	0.1552	Zero order	Zero order
F2*	PEG 6000: PG 50:50	0.9667	-0.5358	0.9727	0.17924	Zero order	Zero order
F3	PEG 6000: PG 40:60	0.9827	-0.5174	0.9799	0.248	Zero order	Zero order
F4*	PEG 6000: PG 20:80	0.9476	-0.4932	0.9923	0.3381	Zero order	Zero order
F4 SD.	PEG 6000: PG 20:80	0.98832	-0.52925	0.984407	1.513	Zero order	Zero order
F5	PEG 6000: PEG 600 60:40	0.9955	-0.5564	0.9462	0.808	Zero order	Zero order
F6	PEG 6000: PEG 600 50:50	0.9985	-0.551	0.9607	0.099	Zero order	Zero order
F7	PEG 6000: PEG 600 40:60	0.9982	-0.544	0.962	0.134	Zero order	Zero order
F8	PEG 4000: PEG 600 60: 40	0.985	-0.547	0.9323	0.1177	Zero order	Zero order
F9	PEG 4000: PEG 600 50:50	0.999	-0.5403	0.9581	0.1507	Zero order	Zero order
F10	PEG 4000: PEG 600 40:60	0.9957	-0.5223	0.9603	0.2244	Zero order	Zero order
F11*	PEG 6000: PEG 1500 30:70	0.9603	-0.503	0.9816	0.3029	Zero order	Zero order
F12	PEG 6000: PEG 1500 : water 50:30:20	0.9601	-0.431	0.8829	0.3025	Zero order	Zero order
F13	PEG 4000: PEG 1500 25:75	0.9649	-0.424	0.8818	0.3199	Zero order	Zero order
F14	Gelatin: Glycerin: Water 14:46:40	0.9216	-0.4592	0.9102	0.2123	Zero order	Zero order
F15	Gelatin: Glycerin: Propylene glycol: Water 14:26:20:40	0.9372	-0.4393	0.9083	0.2818	Zero order	Zero order
F16	Gelatin: Glycerin: Propylene glycol: Water 14:16:30:40	0.9434	-0.4175	0.9102	0.2123	Zero order	Zero order
F17*	Gelatin: Glycerin: Propylene glycol: Water 14:6:40:40	0.9242	-0.3991	0.9268	0.38367	Zero order	Zero order
F17 SD.*	Gelatin: Glycerin: Propylene glycol: Water 14:6:40:40	0.8808	-0.0003	0.8915	0.932938	Higuchi Diffusion K= 8.2706	Zero order

*Schwartz slope was deviated from 0.5.

Table (6): Release characteristics of KT from fatty and emulsion bases suppository formulations.

Code	Suppository composition	Zero Order R^2	First order R^2	Higuchi Diffusion model R^2	K Value (mg ml^{-1} min^{-1})	Order of release	Order of release After Schwartz slope was deviated from 0.5.
F18	Cocoa butter	0.9887	- 0.5428	0.8425	0.0393	Zero order	Zero order
F19	Suppocire CM	0.9887	- 0.4829	0.8459	0.0859	Zero order	Zero order
F20	Suppocire AM	0.9786	-0.4679	0.8829	0.1639	Zero order	Zero order
F20 SD.*	Suppocire AM	0.89735	-0.51418	0.9837	0.294624	Higuchi Diffusion K= 3.9346	Zero order
F21	Witepsol H15	0.9898	-0.4729	0.8812	0.1384	Zero order	Zero order
F22	Cocoa butter: Sodium alginate: Tween 20: Distilled water 74:2:4:20	0.8886	-0.4512	0.8755	0.2474	Zero order	Zero order
F23	W15: W75: Tween 20: Span 60 : PEG 1500: Propylene glycol 24: 10: 5: 1: 40: 20	0.8996	-0.4374	0.8906	0.2946	Zero order	Zero order
F23 SD.*	W15: W75: Tween 20: Span 60 : PEG 1500: Propylene glycol 24: 10: 5: 1: 40: 20	0.84824	-0.48198	0.9623	0.39604	Higuchi Diffusion K= 5.4734	Zero order
F24	W75: Sodium CMC: Tween 20 : Distilled water 50: 1: 4: 45	0.9158	-0.4549	0.8861	0.2295	Zero order	Zero order

*Schwartz slope was deviated from 0.5.

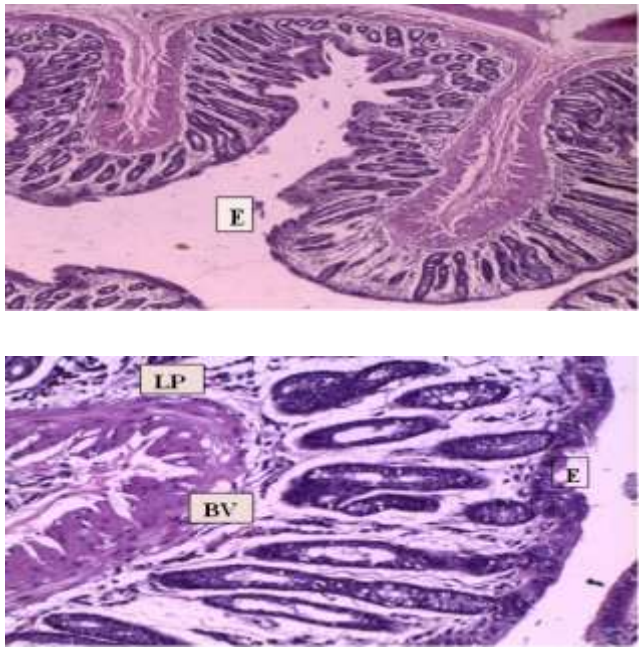


Figure 14: Shows rabbit's rectal mucosa a- after the treatment with plain (F4) PEG base and b- after the treatment with medicated (F4) SD. PEG base.

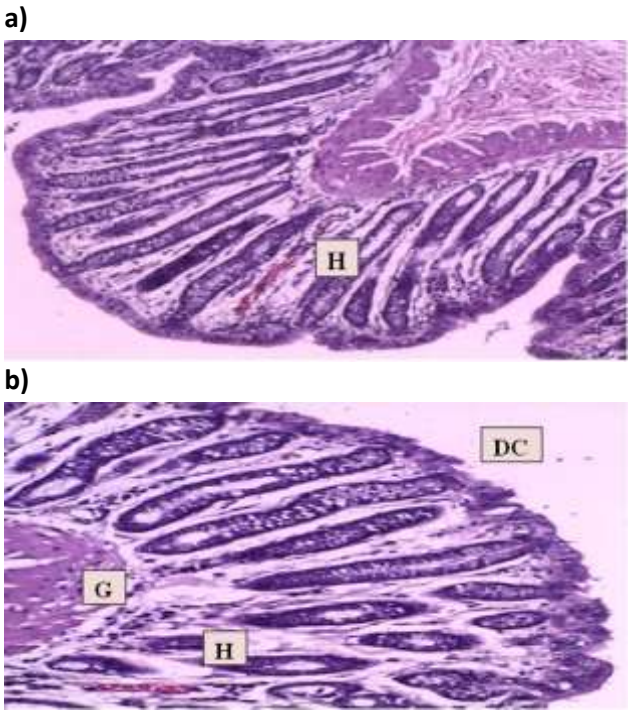


Figure 15: Shows rabbit's rectal mucosa a- after the treatment with plain (F17) glycerin base and b- after the treatment with medicated (F17) SD. glycerin base.

(Figure 13) shows the normal rectal mucosa with its normal mucosal folds in which the simple columnar surface epithelium facing the lumen. The mucosal crypts are lined up in parallel and their mouths are open to the lumen. A clear lamina propria that consists of loose connective tissue surrounds the mucosal glands and extends from the surface epithelium to the smooth muscle cells of the muscularis mucosa [48].

(Figure 14a) shows the rectal mucosa after the treatment with plain (F4) PEG base. The lining epithelium exhibited an increase in the cell height (E) and displacement of nuclei towards the apical surfaces of the cells. The lamina propria (LP) showed few cell infiltration and slight oedema at its lower parts. However, there is no marked loss in the surface epithelial lining. These results can be attributed to the dehydrating effect of the base resulting in water withdrawal towards the lumen. Similar results were also found by Reid et al., [49] while working on Wister rats.

(Figure 14b) shows the rectal mucosa after the treatment with (F4) PEG base containing the solid dispersion of KT [(F4) SD.]. The mucosa exhibited an increase in the activity of mucous glands (G) which indicated by an increase in the size of its lining mucous cells compared with Figure (14 a). The blood capillaries (C) in the lower part of the lamina propria (LP) showed a slight dilatation of blood capillaries (BV) associated with few eosinophil and plasma cell perivascular cell infiltration [50-52]. The mucous glands may participate in the drug absorption process from the rectal lumen. The dilatation in capillaries is also a sign of this absorption process [53].

(Figure 15a) shows the rectal mucosa after the treatment with plain (F17) glycerin base. Irritation and distortion of the lining of the rectal mucosa with marked infiltration of neutrophils can be noticed. Also, hyperemia of the rectal mucosa with minimal amounts of hemorrhage (H) and mucus discharge has been found [54]

(Figure 15b) shows the rectal mucosa after the treatment with (F17) glycerin base containing the solid dispersion of KT [(F17) SD.]. The changes were similar to those shown in **(Figure 15a)** with increase in the activity of mucous glands which may be attributed to absorption process.

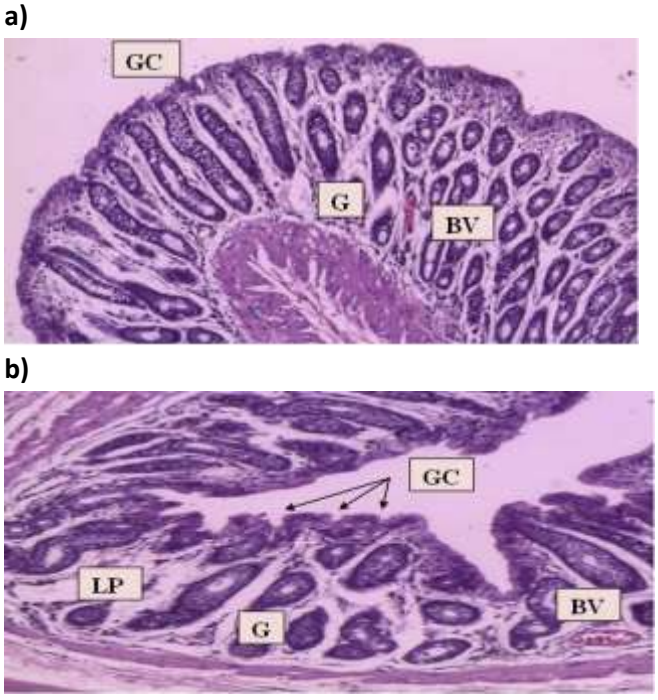


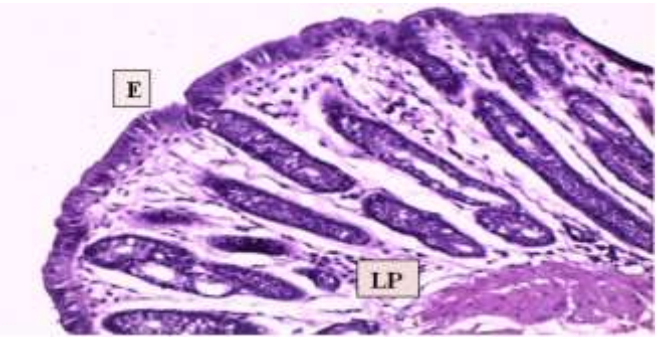
Figure 16: Represents the effect on rabbit's rectal mucosa by a- plain fatty base (F20) and b- medicated fatty base (F20) SD.

(Figure 16a) shows the rectal mucosa after the treatment with plain (F20) suppocire AM suppositories. An increase in the number of goblet cells and mucous glands (G) with slight perivascular cell infiltration around blood capillaries were

observed. The changes observed cannot be considered as damage to the rectal mucosa. [48].

(Figure 16b) shows the rectal mucosa after the application of medicated suppositories [(F20) SD]. The changes were similar to those shown in **(Figure16a)** with slight edema and perivascular cell infiltration of lamina propria (LP).

a)



b)

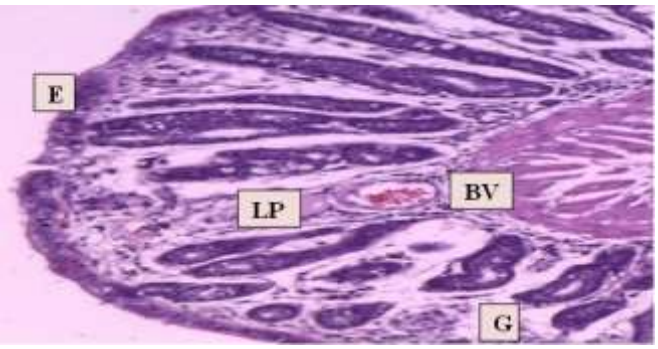


Figure 17: Represents the effect on rabbit's rectal mucosa by a- plain emulsion base (F23) and b- medicated emulsion base (F23) SD.

As can be shown from **(Figure 17a)** and **(Figure 17b)**, representing the effect of plain emulsion base (F23) and medicated [F23 SD.] respectively, on the rectal mucosa. They exhibited the same appearance as that observed with PEG base as they contain PEG 1500 in their composition in addition to Witepsol H_{15} and Tween surfactant which was reported to induce histological changes in the rectal mucosa. These changes are attributed to their dehydrating effect and may result in enhancement of rectal permeability [11].

4. Conclusion:

In conclusion, hydrophilic bases were preferable than lipophilic bases in terms of their ability to release KT from the suppository formulations. Within the different bases used, water soluble PEG base (F4), water soluble gelatin base (F17), fatty base (F20) and emulsion base (F23) gave the highest drug release rate and they were selected as the bases of choice. Solid dispersion of **KT / hydroxypropyl-β-cyclodextrin** at ratio of **(1:7)** in the form of rectal suppository (F4) composed of **20 % of PEG 6000** and **80 % of PG** was found to have a higher in vitro drug release rate. Also, it exhibited the **least** effects on rabbit's rectal mucosa. These results confirm the potential of KT solid dispersion in suppository dosage form, containing (1mg) of KT, as a viable alternative to oral dosage forms for the safe and efficient management of the chronic asthma especially for children asthmatic patients when taken twice daily.

Declaration of Interest

Authors declare that there's no financial/personal interest or belief that could affect their objectivity, or if there is, stating the source and nature of that potential conflict.

Also the authors confirm that the validity of research, not be influenced by a secondary interest, such as financial gain.

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