

Full Length Research Paper

Formulation and evaluation of novel mucoadhesive ketorolac tromethamine liquid suppository

Esmail Mohamed Ramadan, Thanaa Mohamed Borg* and Maha Osama Elkayal

Department of Pharmaceutics, Faculty of Pharmacy, Mansoura University, Egypt.

Accepted 13 October, 2019

Ketorolac tromethamine (KT) loaded mucoadhesive liquid suppository was prepared as a site-specific mucoadhesive rectal dosage form. Poloxamer mixture formed of 21% P407 and 9% P188 were used as liquid suppository base. *In-vitro* release rate of KT from liquid suppository was studied and compared to that from conventional suppository. The safety of the prepared suppository on GIT was conducted, hepatotoxicity of KT after 5 days of administration of liquid suppository was evaluated histologically and biochemically. The levels of liver enzymes alanine aminotransferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were used as the biochemical markers of liver damage. The results obtained revealed that the addition of KT increased the gelation temperature of poloxamer gel and reduced the gel strength and the mucoadhesive force. The study of the release rate of KT from liquid suppository was significantly higher than from conventional suppositories. Histological pictures of the GI tissues indicated no pathological damage after 5 days of rectal administration compared to oral administration. Also, it was revealed that no hepato-cellular damage occurred after administration of liquid suppository; unlike oral administration, which produced certain hepato-toxicity. The administration of KT liquid suppository did not significantly increase the basic levels of ALT and AST when compared to the control. On the other hand, the administration of KT oral solution in a dose of 10 mg/kg body weight/day for 5 days significantly increased serum ALT and AST levels, thus, KT liquid suppository in poloxamer gel was a convenient, safe and effective rectal dosage form for administration with lower hepato-toxic effect.

Key words: Liquid suppository, poloxamer, ketorolac tromethamine, safety, histological study.

INTRODUCTION

Ketorolac tromethamine (KT) is a widely used NSAID for the management of moderate to severe post-operative pain. It has been previously formulated in oral and parenteral route and administered in a dose of 10 mg followed by 10 to 30 mg every 4 to 6 h as required (Catapano, 1996). The most pronounced adverse effects associated with its use are mainly gastrointestinal. These include gastric irritation, mucosal injury, bleeding and perforation which can be fatal, at any time, with or without warning symptoms or a previous history of serious gastrointestinal

events (Vitalae et al., 2003; Chauhan et al., 2001). Furthermore, KT has been associated with a small but significant incidence of hepatotoxicity which ranged from borderline elevations of one or more liver tests to hepatitis, liver failure and cholestatic jaundice that may occur with continued therapy (Elliot et al., 2007). These abnormalities may progress, may remain essentially unchanged or may be transient with continued therapy. Thus, KT should be used with caution in hepatic impairment and should be withdrawn if clinical symptoms of liver disease develop (Catapano, 1996). This suggests that KT-associated hepatotoxicity might be more common than previously recognized and has to be monitored. The previously mentioned adverse effects accompanying the use of oral and parenteral KT gave rise to new ideas for the formulation of KT in rectal form to produce an appealing dosage form for repeated use post-operatively in case of children and elderly, since the rectal route is the most convenient dosage route for unconscious patients (de

*Corresponding author. E-mail: mborgun@Yahoo.com. Tel.: +020502246253/3562. Fax: +020502247496.

Abbreviations: ALP, Alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; KT, ketorolac tromethamine; LDH, lactate dehydrogenase; NSAID, nonsteroidal antinflammatory drug; P, poloxamer.

Boer and Breimer, 1997). However, KT conventional suppositories may exhibit some disadvantages making it not the ideal dosage form for formulation. Rectal administration of KT conventional suppositories may cause local irritation and mucosal damage due to the acidic property of KT that can cause rectal bleeding on administration (Hermann, 1995). Furthermore, there may be some difficulty with repeated and daily administration of conventional KT suppositories. This may lead to the feeling of alien, discomfort and refusal thus, lowering patient compliance (Choi et al., 1998). Thus, to solve the problems of conventional suppository, an attempt was made to develop an intelligent formula, mucoadhesive poloxamer-based liquid suppository (Choi et al., 1998). Liquid suppository exhibits a thermoreversible property, it exists as a liquid at room temperature and turns into a gel instantly at physiological temperature (Choi et al., 1999). Also, mucoadhesive polymers adhere the suppository to the rectal tissues which prevents its migration to the end of the colon. In this study, a mixture of poloxamer 407 (P407) and poloxamer 188 (P188) were selected as a base of liquid suppository (Yong et al., 2004). Poloxamers are known to have mucoadhesive force (Kim et al., 1998), low toxicity (Yun et al., 1999), good drug release characteristics (Miyazaki et al., 1998) and compatibility with other chemicals. Thus, mucoadhesive KT-loaded poloxamer-based liquid suppository was formulated. The physicochemical properties and the release of KT from poloxamer gels were performed. Furthermore, the effect of KT liquid suppositories on the GIT was monitored in rabbits. Hepatotoxicity was also monitored by quantitative analysis of liver enzymes in addition to histological investigation of liver tissues.

MATERIALS AND METHODS

KT was kindly supplied from EIPICO Co., 10th of Ramadan City, Egypt. Poloxamers (P407 and P188) were from Sigma Aldrich, Germany. All other chemicals are of analytical grade. UV-Vis. Spectrophotometer (UV-1601PC, Shimadzu, Japan). Thermostatically- controlled shaking water bath (Grant instrument Cambridge Ltd., Barrington Cambridge, B2, 5002, England). Digital circulating water bath (Polyscience, type 9101, USA). Light microscope with digital camera (Euromex, Netherland).

Preparation of KT liquid suppositories

Poloxamer-based liquid suppositories were prepared by the cold method described by Choi et al. (1998). 10 mg of KT was dissolved in the calculated amount of distilled water at room temperature. The mucoadhesive polymers were incorporated in KT solutions. P407/P188 was slowly added with continuous agitation using a magnetic bar. The mixtures were left at 4°C in refrigerator until clear solutions were obtained.

Evaluation of KT liquid suppositories Measurement of gelation temperature

Gelation temperature was assessed using the tube tilting method (Pisal et al., 2004). 2 ml aliquot of gel was transferred to test tubes,

immersed in a water bath at 4°C and sealed with aluminum foil. The temperature of water bath was increased in increments of 1°C and left to equilibrate for 5 min at each new setting. The samples were then examined for gelation, which was said to have occurred when the meniscus would no longer move upon tilting through 90°C.

Measurement of gel strength

The gel strength was determined according to the method adopted by Kim et al. (1998). 50 g of liquid suppository was put in a 100 ml graduated cylinder and gelled in a thermostat at 37°C. The apparatus for measuring the gel strength (weight 35 g) was then placed into the liquid suppository. The gel strength was determined by the time in seconds the apparatus took to penetrate 5 cm down through the gel.

Determination of the mucoadhesive force

The mucoadhesive force, the detachment stress of the liquid suppositories was determined using a modification of the mucoadhesive force-measuring device used by Choi et al. (2000). A section was cut from the fundus of rabbit rectum and instantly secured with the mucosal side out into each glass vial. The vials were stored at 36.5°C for 10 min. 1 vial connected to the balance and the other fixed with the poloxamer gel added and the height adjusted so that the gel is placed between the mucosal sides of both vials. Water from a burette was allowed to fall in a beaker at a constant rate of 10 mg/sec. Increasing weight of water added gradually would detach the 2 vials. Mucoadhesive force, the detachment stress (dyne/cm²), is determined from the minimal weights of water that detached the 2 vials.

Dissolution test

Dissolution test was carried out using USP dissolution apparatus. 5 g KT liquid suppositories was placed in a semi-permeable membrane tube. Both sides of the tube were firmly tied at its end to prevent leakage. It was then placed in dissolution tester at 36.5°C at 100 rpm using 400 ml phosphate buffer (pH 6.8) as the dissolution medium. At predetermined intervals, 5 ml samples were withdrawn and filtered. 5 ml of fresh buffer were added to the dissolution medium. The drug content of each sample was determined spectrophotometrically at 324 nm.

Analysis of drug release data

The statistical analysis were performed using student's unpaired 't' test. The release mechanism was analyzed using the exponential equation presented by (Peppas, 1985). $Mt/M = k t^n$

$\log Mt/M = \log k + n \log t$

Where Mt/M = fraction of drug released at time t ; k = release constant incorporating structural and geometric characteristics of the drug/polymer system; n = release exponent indicative of the release mechanism.

In-vivo tests on rabbits

The animal experiments were performed according to the ethical regulation of world medical association declaration of Helsinki. 18 male rabbits (1 - 1.5 kg) were divided into 3 groups, each comprised of 6 rabbits. Group A was used as control. Group B was administered KT liquid suppositories at 1.5 g gel/kg into the rectum 4 cm above the anus through a stomach sonde needle. Group C was

Table 1. Physicochemical properties of the KT loaded in Poloxamer mixture with and without mucoadhesive polymers.

Formulatings	Polymer (%w/w)	Gelation temperature (°C)	Gel strength (sec)	Mucoadhesive force (x 10 ² dyne/cm ²)
P407 - P188*	0	18	4.03	3.5
KT-P407 - P188	0	34.5	3.1	1.72
	0.2	34.5	4.6	16.047
KT- P407 - P188- MC	0.6	32.5	16	25.788
	1	32.5	25	35.98
	0.2	33.5	6.5	8.413
KT- P407 - P188- Alg	0.6	31.5	29.5	47.213
	1	27	65.5	77.06

*Poloxamer mixture composed of 21% P407 and 9% P188.

was given oral KT solution in a dose 10 mg/kg through a syringe. The formulae were administered once daily for a period of 5 days.

Biochemical assay of hepatotoxicity

2 ml blood samples were collected from the rabbit marginal ear vein, transferred to test tubes and allowed to stand for 30 min to clot before centrifugation at 3000 rpm for 10 min. Serum was separated and stored at -20°C, for subsequent analysis of liver enzymes. The levels of ALT, AST, ALP and LDH were used as the biochemical markers of liver damage. The enzyme levels were expressed in international units per liter (IU/L) (Caglar and Kolankaya, 2007).

Histological examination of rabbit liver and GIT

The animals were sacrificed, rabbit liver, stomach and rectum were isolated, rinsed with saline solution and stored in 10% carbonate-buffered formalin, embedded in paraffin wax and cut into sections of 3 µm thick. The sections were stained with hematoxylin-eosin (Hx and E) and examined under light microscope (Miyazaki et al., 1998).

RESULTS AND DISCUSSION

Evaluation of KT liquid suppositories

Measurement of gelation temperature Poloxamer mixture form of 21% P407 and 9% P188 was selected as the system of choice for the liquid suppository base since it exhibits the optimum gelation temperature in the desirable range (Ramadan et al., 2008). KT is added in concentration of 10 mg and was kept fixed throughout the experiments.

Table 1 illustrates the physicochemical properties of poloxamer gel. It was found that 10 mg KT dramatically raised the gelation temperature of the gel from 18 to 34.5°C. This was in agreement with the results obtained by Gilbert et al. (1987) who stated that water-soluble substances cause modification of the process of micellization of poloxamer solutions, leading to an mucoadhesive KT liquid suppositories with modulated gelation temperature, gel strength and mucoadhesive increase in the gelation temperature. In order to develop force, mucoadhesive

polymers were added in different concentrations (Shin and Kim, 2000). It was found that upon the addition of 0.2% MC and Alg., the gelation temperature of poloxamer mixtures were 34.5 and 33.5°C. Increasing the concentration to 0.6% reduced the gelation temperature from 34.5 to 32.5 and 31.5°C, respectively. Also, the effect of bioadhesive polymers was more obvious as their concentrations increased to 1%. The addition of 1% MC and Alg. reduced the gelation temperature of poloxamer solutions to 32.5 and 27°C, respectively. It could be concluded that, the addition of mucoadhesive polymers generally reduced the gelation temperatures. This was in good agreement with that reported by Choi et al. (1998). The reduction was mainly concentration-dependent. This was in accordance with the results obtained by Kim et al. (1998). The gelation temperature-lowering effect of the mucoadhesive polymers used could be explained by their ability to bind to the polyoxyethylene chains of the poloxamer molecules, which promotes dehydration and causes an increase in the entanglement of adjacent molecules with more extensive intermolecular hydrogen bonding, thus producing gelation at lower temperature (Gilbert et al., 1987). Block copolymer poloxamer gel is thought to be formed by hydrogen bonding in the aqueous system, caused by the attraction of the poloxamer ether oxygen atom to a proton of water. If the hydrogen bonding is supplemented by adding compounds with hydroxyl group, the gelation temperature decreases (Malmsten and Lindman, 1992).

The higher mucoadhesion of alg than MC may be attributed to the mechanical properties of the gel, added to modifications that would produce on cellular surfaces.

Measurement of gel strength

In the development of liquid suppository, the gel strength is important in finding the condition that allows the easy insertion of suppositories and no leak from the anus. At high gel strength, it is difficult to insert the suppositories. On the other hand, at low gel strength the suppositories leaked from the anus (Choi et al., 1998). The addition of KT decreased the gel strength from 4.03 to 3.1 s. This

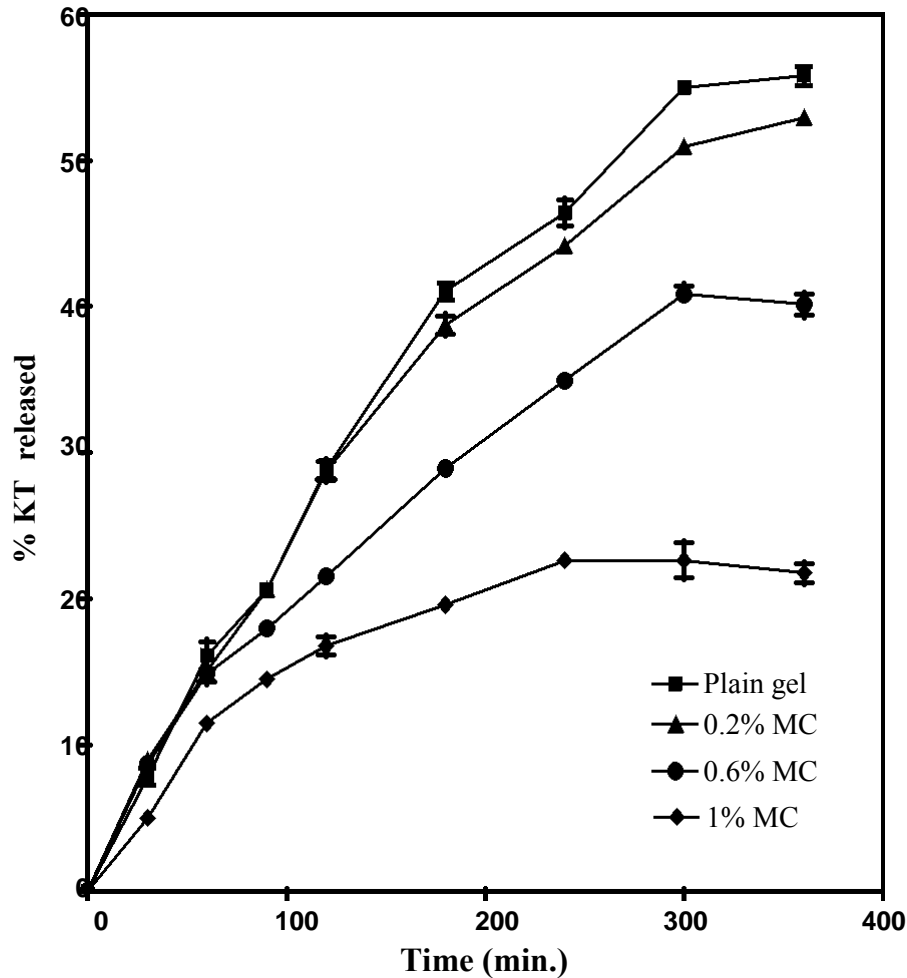


Figure 1. Effect of MC concentration on the release of KT from liquid suppositories in phosphate buffer pH 6.8.

can be explained on the basis that KT was incorporated into the gel matrix leading to weakening the binding force of the cross-linked reticular structure of poloxamer gel. It was found that, the addition of MC or alg. increased the gel strength of poloxamer mixture in a concentration-dependent manner.

The large increase in gel strength caused by the addition of alg might be attributed to the strong cross-linking bonding of sodium alg with the cross-linking reticular poloxamer gel forming more closely packed micelles (Yong et al., 2001). Another postulation was presented by Hongyi et al. (2006) who stated that, the polymers with hydrophilic groups as the carboxyl and hydroxyl groups can bind strongly to the oligosaccharide chains by forming hydrogen bonds, electrostatic attraction or hydrophobic interaction, resulting in increase in gel strength.

Measurement of the mucoadhesive force

The incorporation of KT into poloxamer gel reduced the mucoadhesive force from 3.5 to 1.72×10^2 dyne/cm². This can be explained by the incorporation of KT into the

gel matrix leading to weakening the binding force of the cross-linked reticular structure of poloxamer gel. The addition of mucoadhesive polymers generally reinforced the mucoadhesive force of liquid suppositories. This effect mainly depends on the nature and the concentration of the polymer (Kim et al., 1998).

The mucoadhesive force-enhancing effect of MC can be explained based on the theory postulated by Liu and Chu (2000). Cellulose derivatives having many hydroxyl groups promote dehydration of poloxamers and consequently the hydrophobic interactions between the PPO blocks. The mechanism of the mucoadhesion enhancing effect of different polymers might be related to hydrogen bonding between the liquid suppository base and the mucosal membrane (glycoprotein) via carboxyl groups in the mucoadhesive polymers (Lehr et al., 1992).

In-vitro release of KT from liquid suppositories

The effect of mucoadhesive polymers on the release of KT from liquid suppository was illustrated in Figure 1 and 2. It was found that KT was released from poloxamer gel

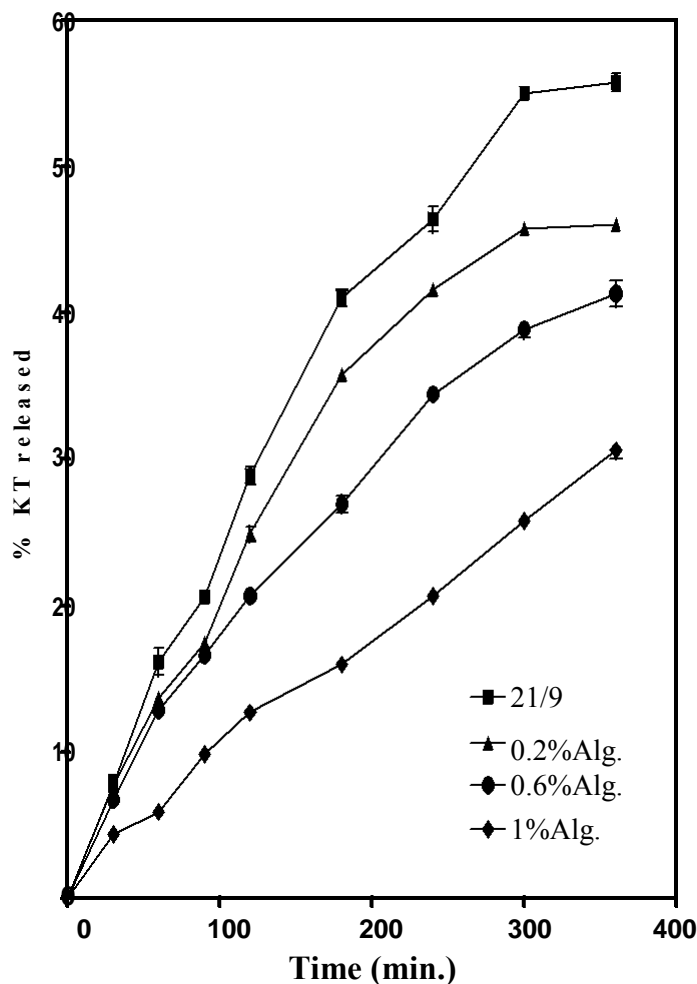


Figure 2. Effect of sodium alginate concentration on the release of KT from liquid suppositories in phosphate buffer pH 6.8

Table 2. Release kinetics of KT from different KT liquid suppositories.

Formulations	Polymer (%w/w)	Release Exponent (n)	Kinetic constant (K)	Correlation Coefficient (r^2)
KT-P407 - P188	0	0.8055	0.5086	0.9828
KT- P407 - P188- MC	0.2	0.7418	0.7659	0.9905
	0.6	0.6340	1.0387	0.9926
	1	0.5046	0.9986	0.9775
KT- P407 - P188- Alg	0.2	0.7596	0.5897	0.9816
	0.6	0.7512	0.5430	0.9959
	1	0.8065	0.2358	0.9884

at 55.5% after 6 h. The addition of MC in concentration of 0.2, 0.6 and 1% reduced the % KT released to 53, 40 and 21.5% respectively. Such retardation in the release rate can also be contributed to increasing the concentration of swellable polymers increased the product viscosity and thereby decreased the rate of penetration of the dissolution medium. This was in agreement with that reported by (Kim et al., 1998). It could be concluded that, the addition of different mucoadhesive polymers in dif-

ferent concentrations retarded the release of KT from liquid suppositories. This mainly depended on the viscosity of the polymer added and its concentration.

Analysis of drug release data

The kinetic parameters are shown in Table 2. From the data illustrated it was found that most formulae exhibited n values between 0.5 - 1 indicating a non-Fickian or ano-

(A) Control; (B) liquid suppositories and (C) oral solution.

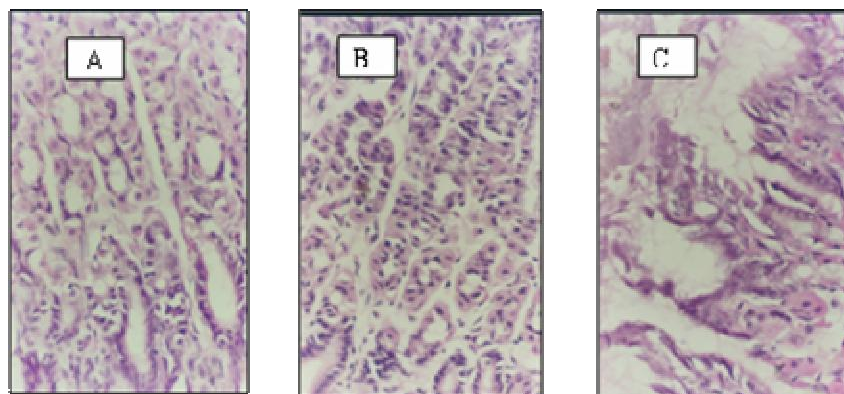


Figure 3. Morphology of stomach of rabbits after administration of KT liquid suppositories and oral solution

Table 3. Effect of KT administration on the hepatic marker enzymes of control and experimental rabbits.

Parameter (IU/L)	Group A	Group B	Group C
ALT	45 ± 8.03	49 ± 11.9	80 ± 5.84
AST	35 ± 2.69	38 ± 7.27	68 ± 3.91
ALP	109.4 ± 1.03	97 ± 0.94	165 ± 0.43
LDH	284.34 ± 7.12	297.25 ± 12.38	314.69 ± 7.2

KT is administered at 10 mg/kg body weight/day for 10 days. Group A (control group), group B (KT liquid suppository group) and group C (KT oral solution group). Values are expressed as mean ± SD, n = 6.

malous release which could be due to gel erosion and diffusion mechanism. On the other hand, at 1% MC, the *n* value was found to be close to 0.5 indicating that KT might be released by Fickian diffusion (Higuchi model). This was in agreement with the results reported by Suh and Jun (1996) who studied the release profile of naproxen from P407 gels. Highly linear responses ($r > 0.999$) indicated that the release of naproxen from the P407gel was in full compliance with the Higuchi equation. The release of KT was found to be through extra-micellar aqueous channels of the gel matrix which means the outer layer of poloxamer cross-linking system (poloxamer micelle) (Veyries et al., 1999).

Safety test of GIT in rabbits

It was clearly observed that, the gastric mucosa of rabbits of group B given KT liquid suppositories was generally found to be normal resembling that of control Figure 3b). The mucosa exhibited normal thickness with normal arrangement of cells but with slight edema in between the epithelial cells, in addition to wide spread inflammatory cells in the field together with hyperplasia of oxyntic cells (hydrochloric acid-secreting cells) to some extent. In contrast, the gastric mucosa of rabbits of group C given

oral KT solution showed marked ulceration in some areas, as indicated by the complete disappearance of mucosal cells together with numerous oxyntic cells and inflammatory cells. In addition, hemorrhage was observed in between the cells as well as cellular degeneration in some areas Figure 3c).

The gastric lesions produced in liquid suppository group could be due to the systemic effect of the drug on PG synthesis, since KT is an inhibitor of prostaglandin (PG) synthesis. PGs play a role in protecting the gastric mucosa from acidic secretions. Thus, inhibition of PG synthesis by KT reduces this protective effect and increases the risk of gastric ulceration (Fiedler, 1997). However, the extensive gastric damage induced by oral administration of KT in rabbits of group C could be attributed to the combined mechanisms, local damage caused by direct contact of the acidic drug moiety with the gastric wall in addition to the systemic damage caused by the inhibition of PGs synthesis (Traversa et al., 1995).

Figure 4b illustrate the rectum of rabbits of liquid suppository group. It was generally observed that the epithelium was normal having typical goblet cells and normally arranged crypts but with few numbers of inflammatory cells in the coreum and widely separated crypts

(A) Control; (B) liquid suppositories and (C) oral solution.

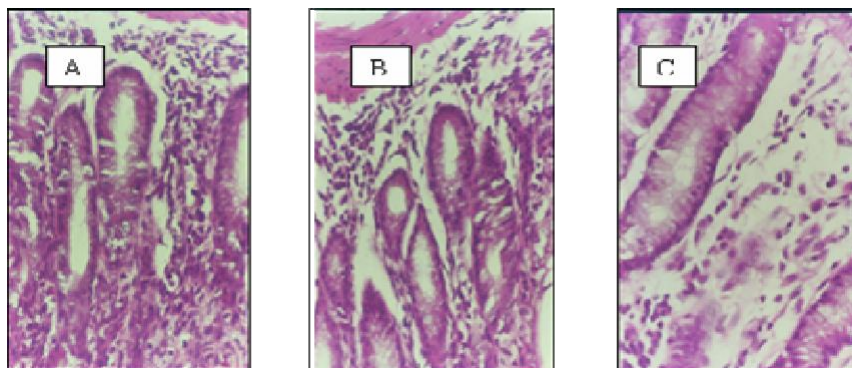


Figure 4. Morphology of rectal tissue of rabbits after administration of liquid suppositories and oral solution

(edema) in between the epithelial cells. The histological changes observed might be due to the local effect of KT on the rectal wall in addition to the mechanical irritation produced by the stomach sonde needle used in the administration of the drug for 5 days. However, the changes observed in the rectal wall could be said to be mild not severe. The rectum of rabbits administered KT oral solution is illustrated in Figure 4c. Well observed edema with a large number of inflammatory cells in the epithelial cells was seen in addition to focally lost mucosal layer in some areas indicating ulcer formation.

Study of liver hepatotoxicity of KT

Biochemical assay of hepatotoxicity

Table (3) illustrates the effect of KT administration on the hepatic marker enzymes of control and experimental rabbits. It was observed that, the administration of KT liquid suppository did not significantly increase the basic levels of ALT and AST (49 and 38 IU/L, respectively) when compared to the control, which showed basic serum ALT and AST levels of 45 and 35 IU/L, respectively. On the other hand, the administration of KT oral solution in a dose of 10 mg/kg body weight/day for 5 days (group C) significantly increased serum ALT and AST levels to 80 and 68 IU/L, respectively. Concerning serum ALP level, it was observed that the level of ALP in group B showed slight reduction in ALP level (97 ± 3 IU/L) when compared to control group (A) (109.4 ± 2 IU/L). While, ALP level was significantly increased after the administration of KT oral solution (group C) to 165 ± 3 IU/L. The administration of KT in liquid suppository and oral solution produced mild effect on the level of LDH, a marker of cellular damage. Serum LDH level was moderately elevated in groups B and C to some extent (297.25 and 314.69 IU/L, respectively) compared to group A (284.34 IU/L). But it was clearly observed that the increment was to a lower extent than that in transaminases. Determination of se-

rum ALT and AST is one of the most frequently performed assays, together with ALP and LDH, as an aid in the diagnosis of hepatotoxicity (Kang et al., 2008). The increase in the level of serum ALT and AST could be expected to occur associated with pathology involving necrosis of the liver. In the liver, ALT and AST are significantly increased in such cases and escape to the plasma from the injured hepatic cells (Fiedler, 1997). The increase in ALP level in serum mostly reflects liver damage, change in membrane permeability and marked liver injury (Deepa and Varalakshmi, 2003). An increase in the level of circulating LDH is an index of parenchymal liver damage and even hepatitis. This might be due to the release of these enzymes from the cytoplasm into the blood circulation rapidly after rupture of the plasma membrane and cellular damage (Injac et al., 2008).

The previous findings demonstrate that oral KT administration exhibited certain hepatotoxic effect as detected by elevated liver enzymes. It can be the cause of hepatocellular damage and thus increase the production of the liver enzymes and hence increase their release to serum. On the contrary, the rectal administration of KT liquid suppositories exhibited reduced hepatotoxicity as indicated by near normal levels of liver enzymes.

Histological examination of rabbit liver

To support the results of biochemical assay, the histological study of liver tissues was carried out. Figure 5 shows the results of histological observations for rabbit liver tissues administered KT liquid suppository and oral solution.

In the liver of rabbits given KT liquid suppositories, mild changes were observed which took the form of inflammatory changes with some abnormality in the cellular architecture as illustrated in Figure 5b. Normal liver tissues were observed having typical hepatocytes with normal shape and size with regular lobular arrangement. In contrast to this, the livers of rabbits of group C showed

(A) Control; (B) liquid suppositories and (C) oral solution.

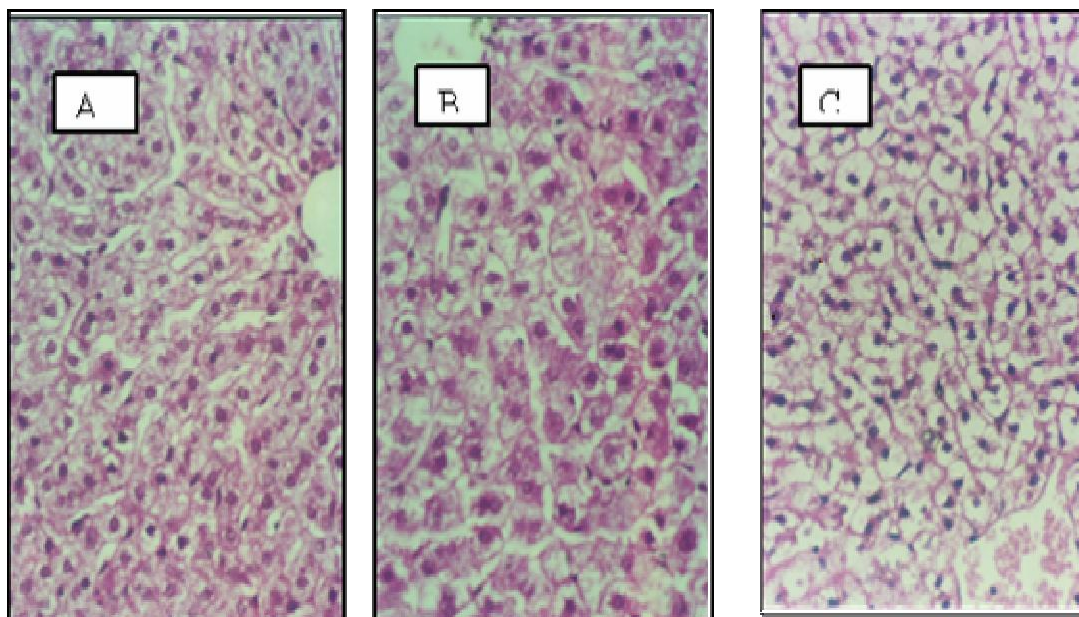


Figure 5. Morphology of liver tissue of rabbits after administration of liquid suppositories and oral solution.

extensive hepatocellular damage, which may progress to total liver failure, Figure 5c shows totally degenerated hepatocytes where the cells appear as ghosts, having dark pyknotic nuclei with loss of architecture, which is indicative of hepatic failure.

From the previous observations it can be concluded that the changes caused in rabbit liver of group C were more pronounced and serious than those produced in group B administered KT liquid suppositories. These histological findings support the results obtained by biochemical assay of rabbit serum, previously described which detected that rectal administration of KT in liquid suppository form produced insignificant alterations of liver function tests in rabbit serum, unlike the oral administration of KT aqueous solution which produced marked elevations of liver enzymes.

Conclusion

KT could be prepared in mucoadhesive liquid suppository. It provides significant safety to the GIT, producing minimal harm to the stomach and rectum. In addition, the liver tissue was not affected seriously by the prolonged use. KT-loaded poloxamer-based liquid suppository can be used as a safe, mucoadhesive and effective rectal dosage for clinical application.

REFERENCES

Caglar S, Kolankaya D (2008). The effect of sub-acute and sub-chronic exposure of rats to the glyphosate-based herbicide Roundup, *Environmental Toxicology and Pharmacology* 25 : 57-62.

- Catapano MS (1996). The analgesic efficacy of ketorolac for acute pain. *J. Emerg. Med.* 14: 67-75.
- Celik I, Suzek H (2008). The hematological effects of methyl parathion in rats *J. Hazardous Mater.* 153: 1117-1121.
- Chauhan RD, Idom CB, Noe HN (2001). Safety of ketorolac in the pediatric population after ureteroneocystostomy, *J. Urol.* 166: 1873-1875.
- Choi HG, Jung JH, Ryu JM, Yoon SJ, Oh YK, Kim CK (1998). Development of in-situ gelling and mucoadhesive acetaminophen liquid suppository, *Int. J. Pharm.* 165: 33-44.
- Choi HG, Kim CK (2000). Development of omeprazole buccal adhesive tablets with stability enhancement in human saliva, *J. Control. Rel.* 68: 397-404.
- Choi HG, Lee MK, Kim MH, Kim CK (1999). Effect of additives on the physicochemical properties of liquid suppository bases, *Int. J. Pharm.* 190: 13-19.
- de Boer AG, Briemer DD (1997). Hepatic first-pass effect and controlled drug delivery following rectal administration, *Adv. Drug Deliv. Rev.* 28:229-237
- Deepa PR, Varalakshmi P (2003). Protective effect of low molecular weight heparin on oxidative injury and cellular abnormalities in adriamycin-induced cardiac and hepatic toxicity, *Chem. Biol. Interact.* 146: 201-210.
- Elliot VH, Pinto A, Moore PA (2007). Adverse drug reactions involving common prescription and over-the-counter analgesic agents, *Clin. Ther.* 29: 2477-2498.
- Fiedler MA (1997). Clinical implications of ketorolac for post-operative analgesia, *J. Peri Anesthesia Nursing*, 12: 426-433.
- Gilbert JC, Richardson JL, Davies MC, Pakin KJ (1987). The effect of solutes and polymers on the gelation properties of Pluronic F127 solutions for controlled drug delivery, *J. Control. Rel.* 5: 113-118.
- Hermann TW (1995). Recent research on bioavailability of drugs from suppositories, *Int. J. Pharm.* 123: 1-11
- Hongy Q, Li L, Haung C, Li W, Wu C (2006). Optimization and physicochemical characterization of thermosensitive Poloxamer gel containing puerarin for ophthalmic use, *Chem. Pharm. Bull.* 54: 1500-1507.
- Injac R, Perse M, Obermajer N Milic VD, Prijatelj M, Cerar A, Strukelj B (2008). Potential hepatoprotective effects of fullereneol C60(OH)24 in

- doxorubicin-induced hepatotoxicity in rats with mammary carcinomas. *Biomaterials* 29 : 3451-3460.
- Kang JS, Wanibuchi H, Morimura K, Wongpoomchi R, Chusiri Y, Gonzalez. FJ, Fukushima S (2008). Role of CYP2E1 in thioacetamide-induced mouse hepatotoxicity, *Toxicol. Appl. Pharmacol.* 228:295-300.
- Kim CK, Lee SW, Choi HG, Lee MK, Gao ZG, Kim IS, Park KM (1998). Trials of in-situ gelling and mucoadhesive acetaminophen liquid suppository in human subjects, *Int. J. Pharm.* 174:201-207.
- Lehr CM, Bouwatra JA, Bodde HE, and Junginger HE (1992), A surface energy analysis of mucoadhesion: contact angle measurement on polycarbophil and pig intestinal mucosa in physiologically relevant fluids. *Pharm. Res.* 9: 70-75.
- Liu T, Chu B (2000). Formulation of homogenous gel-like phases by phase transition, *J. Appl. Cryst.* 33: 727-730.
- Malmsten M, Lindman B (1992), Self-assembly in aqueous block copolymer solutions. *Macromolecules* 25: 5440-5445.
- Miyazaki S, Suisha F, Kawasaki N, Shirakawa M, Yamatoya K, Attwood D (1998), Thermally reversible xyloglucan gels as vehicles for rectal delivery. *J. Control. Rel.* 56: 75-83
- Peppas NA (1985). Analysis of Fickian and non-Fickian drug release polymers, *Pharm. Acta Helv.* 60: 110 -111.
- Pisal SS, Paradkar AR, Mahadik KR, Kadam SS (2004), Pluronic gels for nasal delivery of vitamin B12. Part I: Preformulation study. *Int. J. Pharm.* 270: 37-45.
- Ramadan EM, Borg TM, Elkayal MO (2008). Formulation of site-specific mucoadhesive liquid suppositories as a method to decrease hepatotoxicity in rabbits. *Assiut J. Pharm. Sci.*, 31:331-344
- Shin SC, Kim JY (2000). Enhanced permeation of triamcinolone acetonide through the buccal mucosa. *Eur. J. Pharm. Biopharm.* 50: 217-220.
- Suh H, Jun HW (1996). Physicochemical and release studies of naproxen from Poloxamer gels, *Int. J. Pharm.* 129:13-20.
- Traversa G, Walker AM, Ippolito FM (1995), Gastrointestinal toxicity of different nonsteroidal anti-inflammatory drugs. *Epidemiology.* 6:49-54.
- Veyries ML, Couarraze G, Geiger S, Agnley F, Massias L, Kunzli B, Faurisson, F, Rouveix B (1999). Controlled release of vancomycin from Poloxamer 407 gels, *Int. J. Pharm.* 192: 183-193.
- Vitale MG, Choe JC, Hwang MW, Bauer RM, Hyman JE, Lee FY, Roye DP (2003), Use of ketorolac tromethamine in children undergoing scoliosis surgery: An analysis of complications. *Spine J.* 3: 55-62.
- Yong CS, Choi JS, Quan QZ, Rhee JD, Kim CK, Lim, S.J., Kim KM, Oh PS Choi HG (2001). Effect of sodium chloride on the gelation temperature, gel strength and bioadhesive force of Poloxamer gels containing diclofenac sodium, *Int. J. Pharm.* 226: 195-205.,.
- Yong CS, Yang CH, Rhee JD, Lee BJ, Kim DC, Kim DD, Kim CK, Choi JS, Choi HG (2004). Enhanced rectal bioavailability of ibuprofen in rats by Poloxamer 188 and menthol, *Int. J. Pharm.* 269: 169-176.
- Yun MO, Choi HG, Jung JH, Kim CK (1999). Development of thermoreversible insulin liquid suppository with sodium salicylate. *Int. J. Pharm.* 189:137-145.