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Article

Pharmacological and toxicological investigations of etodolac loaded gum katira microspheres prepared by $W_1/O/W_2$ emulsion solvent evaporation technique in rats

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Etodolac is a non-steroidal anti-inflammatory drug (NSAID) and approved by USFDA as a COX2 inhibitor. Although etodolac therapy provides clinical benefits, it is associated with upper gastrointestinal (GI) tract complications also. Etodolac loaded gum Katira microsphere (ELGKM) was prepared by $W_1/O/W_2$ emulsion solvent evaporation technique. The gastric irritation properties of orally administered pure etodolac, ELGKM and blank microspheres (without etodolac) were evaluated in experimental rats treated for 6 days. The stomach examination and biochemical investigation of stomach tissue of treated rats indicated that ELGKM formulation remarkably reduced ulcerogenecity as compared to pure etodolac. The anti-inflammatory activities of pure etodolac and ELGKMs were ascertained by the implantation of cotton pellets in rats for 6 days. Based on the results, ELGKMs showed significant anti-inflammatory activities ($P<0.01$) as compared to control group. The cotton pellets test suggested that ELGKM formulation retained more anti-inflammatory properties among the groups. The hematological changes, biochemical analysis and histopathological studies of subacute toxicity in rats revealed that ELGKM were the effective sustained release formulation in the treatment of chronic pain and inflammation. In conclusion, the physicochemical characterization, pharmacological and toxicological studies suggest that ELGKMs may represent as a potential candidate for sustained drug delivery (10-12 hours) in chronic joint pain related diseases with remarkably diminished gastrointestinal side effects.

Keywords: Etodolac/inflammatory conditions. Etodolac/physicochemical characterization. Gum Katira/microspheres. Pain therapy.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) have been used for the treatment of several rheumatic and inflammatory diseases for more than 30 years. Etodolac is used as an analgesic for the treatment of signs and symptoms of rheumatoid arthritis, osteoarthritis and the relief of post-operative pain (Craig, Stitzer, 2004). The pharmacological actions of etodolac are related to inhibition of prostaglandin bio-synthesis at the site of inflammation. Etodolac decreases the synthesis of peripheral prostaglandins involved inflammation by inhibition of the cyclooxygenase (COX-2) enzyme preferentially (Glaser *et al*., 1995).

The oral route is one of the most popular routes of drug delivery because of easy administration, patient compliance, least sterility and flexible design of dosages forms. The most conventional dosage forms (tablets and capsules) for oral drug delivery are formulated to get the active compound in the systemic circulation immediately after administration. Now a day, various modified drug release system have been developed to deliver therapeutic drug at a controlled rate in sustained manner in gastrointestinal tract (Shojaei AH, 1998). Sustained release formulations, ELGKMs were developed by $W_1/O/W_2$ emulsion solvent evaporation technique. The physicochemical evaluation revealed that ELGKMs with high drug entrapment efficiency (70-75%) delivered

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the drug for prolong period of time (10-12 hours) in a sustained fashion. There were no significant chemical interaction among the excipients used in the microspheres preparation (Ruhidas *et al.,* 2016).

Etodolac is well absorbed from the gastrointestinal tract following oral administration. The bioavailability of etodolac is about of 80% and plasma peak concentration usually attained within about 1.4 hours (tablet or capsule) or 7 hours (extended-release tablets) (Indian Pharmacopoeia Commission, 2014). In case of rheumatoid arthritis and osteoarthritis, patients generally forget to take medicine in the morning and evening or in between. Once or twice daily dosing thus improves therapy by maintaining steady state plasma drug concentration of the drug in the blood, so avoids the peaks of high plasma drug concentration as well as troughs of low plasma drug concentration.

Gastrointestinal side effects are the most frequently occurring adverse effects associated with orally administered anti-inflammatory drugs. The risks of gastrointestinal ulceration, bleeding and even perforation with NSAIDs therapy was well known (Rainsford, 1989). These effects may result from local action of drugs which caused injuries to the sub-mucosal capillaries with subsequent necrosis and bleeding or from the inhibition of formation of protective prostaglandins.

ELGKM avoids the side effects associated with high concentration as well as no side effect during troughs, giving better overall therapy and improves patient compliance. Therefore, appreciable amounts of etodolac are prescribed as a special formulation (ELGKMs), which has the potential to minimize its toxicity and improve its pharmacological efficacy as well as bioavailability. ELGKM has been formulated to reduce gastrointestinal tract irritation and to release the drug over a prolonged period (10-12 hours). The present pharmacological and toxicological study has shown reduction in ulcerogenic property and better anti-inflammatory activity of ELGKMs formulation as compared to pure etodolac.

MATERIAL AND METHODS

Chemicals

Etodolac (molecular weight, 287.35g/mol, 95.42%) was gifted by M/S Fleming Laboratory Limited, Dist. Medak, Andhra Pradesh, India. Crude Gum Katira was obtained from Seoni District of Madhya Pradesh. Eudragit®RS75 and Eudragit®RL75 polymer granules were obtained as gift sample from Evonik Rohm, Pharma Polymers, Kirsechenallee, Darmastand, Germany. Span 80 (Loba Chemie Pvt. Ltd, Mumbai, India), Tri-Sodium Orthophosphate (Loba Chemie Pvt. Ltd. India), Hydrochloric Acid 35% (Merck Life Science Pvt. Ltd. India), Tween 80 (Merck Specialties Pvt. Ltd, India), Dichloromethane (Merck Specialities Pvt. Ltd, India), Potassium dihydrogen phosphate (Merck Specialties Pvt. Ltd, India), and all others analytical grade chemicals were purchased and used as received.

Animal

Male albino rats (150-200 g) and rabbits (1.5-2 kg) were taken as experimental animal. The animals were acclimatized to laboratory conditions for 3days before the commencement of the experiment and kept under standard condition of temperature (25 ºC), relative humidity (70±10%) and a 12 hours light/12 hours dark cycle environment. During the study period, guideline of Committee for the Purpose of Control and Supervision of Experiment on Animal (CPCSEA), Institutional Animal Ethics Committee (IAEC) were properly followed for the maintenance of animal and the experiment protocol was approved by Animal Ethics Committee of Jadavpur University, Ref. No. AEC/PHARM/1601/02/2016 dated 22/04/2016.

Preparation of etodolac loaded gum Katira Microspheres

Etodolac loaded gum Katira microspheres were prepared by double-emulsion solvent evaporation technique. Gum Katira (50 mg) was mixed with 4ml of phosphate buffer (pH-6.8) to form a homogeneous mixture in a magnetic stirrer for 1hr at a constant temperature of 40-45^{0}C. Etodolac was added to the homogeneous mixture and stirring was continued for another 1hr. The prepared etodolac and gum Katira mixture was then dispersed in a solution of Eudragit®RS100 and Eudragit®RL100 (7:1), Dichloromethane, Acryflow (Lubricating agent) and Span 80 (30 µL) through a 20-gauge syringe. The above mixture was homogenized well for 5-10 minutes using magnetic stirrer (1000rpm) to form W_1/O emulsion. A separate acidic aqueous solution (100 mL) containing Tween 80 (50 µL) and a slight amount of polyvinyl alcohol was subjected to mechanical stirring (900 rpm) and to it the previously prepared W_1/O emulsion was added drop wise using a 16-gauge syringe to form W1/O/W2 emulsion with a continuous stirring for 2-2.5 hours. The resultant microspheres formed were washed with distilled water followed by air drying for 24 h and final storage in desiccators for pharmacological investigation (Ruhidas *et al.* 2016).

Ulcerogenic investigation

Experimental procedure

Ulcerogenic properties of etodolac and ELGKMs formulation were assessed using the method described by Shanbhag *et al*. (Meier *et al*., 1950). Rats were randomly distributed into four groups (n = 6 in each group). The control group received no drug while the standard groups were given pure etodolac (50 and 75 mg/kg b.w., *po*), test groups received ELGKMs formulation (50 and 75 mg/kg b.w) respectively, *po*) and blank microspheres formulation (equal to 50 mg) for 6 days. The animals were fasted 8 hours pre and 4 hours post-treatment (Vyas et al., 2009). Food and water were available for the rest of the time. On 7th day, the animals were sacrificed. The stomachs were removed and placed on the saline soaked filter paper until the examination. A longitudinal incision along the greater curvature was made with fine scissors. The stomachs were or absences of gastric irritation were determined. Based on the severity of gastric mucosal damage, each gastric lesion was assigned a score. The scores were averaged and the mean score tabulated as the severity indexes for the drug and the formulated drug suspension administered. The rating scale of mucosal damage of stomach tissue has been shown in Table I (Tammara *et al*., 1993). The overall score was divided by the factor of 10, which was designated as the 'Ulcer Index' (Main, Whittle, 1975).

TABLE I - Rating scale of mucosal damage of stomach in rats

Observation of mucosal damage	**Score**
No lesion	**0.0**
Punctiform lesions (less than 1 mm)	**1.0**
Five or more punctiform lesions	**2.0**
One to five small ulcers (1-2 mrn)	**3.0**
More than five small ulcers or one large ulcer	**4.0**
More than one large ulcer (greater than 4 mm)	**5.0**

Preparation of stomach tissue homogenate

After the observation of mucosal damage of stomach of the different groups of animals were kept in So-Low Ultra Low freezer (-80ºC) for 12 hours (So-Low Ultra Low freeze, Model No.- C85-5, USA). The following day, a small portion of stomach tissue was homogenated using a homogenizer (RIMI MOTOR, Type-RQ 127A) with the favorable buffer solution for in different biochemical testing for assay of ulceration.

Biochemical investigation of ulcerogenic stomach of rats

Estimation of Total Protein (T.P)

To 0.1 mL of tissue homogenate 0.5 mL freshly prepared reagent C (alkaline coper solution: 50 mL of reagent A mixed with 1 mLof reagent B) was added and kept for 10 minutes at room temperature. To 0.5 mL of the solution, 0.5 mL of distilled water and 5.0 mL of reagent C was added. Then 0.5 mL reagent D (Folin and Ciocalteu reagent: diluted 2:1 with distilled water) was added to this solution and kept for 30 minutes at room temperature. Optical density was measured at 660 nm in UV. Total Protein was estimated by the Lowery method (Peterson, 1979) using Bovine serum albumin (BSA) as standard, at 660 nm.

Assay of thiobarbituric acid reactive substances (TBARS)

The level of Lipid per-oxidation was estimated by measuring the concentration of malondialdehyde in the stomach tissue according to the modified method of Ohkawa *et al.* (1979). 0.5 ml of tissue homogenate (0.025 M Tris-HCl buffer, PH-7.8),0.5 mL saline and 1.5 mL of 20% TCA (Tricyclic acetic acid) were added and mixed well and then centrifuged at 3000 rpm for 20 minutes. To 1.0 mL of the protein raw supernatant, 1.5 mL of 0.8%TBA (Thiobarbituric Acid) reagent was added. The content mixture was mixed well and boiled for 1 hour at 95 ºC and pink colour developed. The tubes were then cooled under running tap water. The absorbance of clear supernatant was measured against reference blank at 532 nm (Ohkawa, Ohishi, Yagi*,* 1979).

Assay of catalase (CAT)

The stomach tissue homogenate was prepared by using phosphate buffer (0.01 M, PH-7.0). The homogenate was centrifuged at 5000 rpm for 10 minutes. To 0.9 ml phosphate buffer, 0.1ml of tissue homogenate (supernatant) and 0.4 mL of 2 mM H_2O_2 were added. The reaction was arrested after 15, 30, 45, 60 seconds by adding 2.0 mL of dichromate acetic acid mixture (5% potassium dichromate and glacial acetic acid were mixed in 1: 3 ratio). The tubes were kept in a boiling water bath for 10 minutes and cooled. The colour was developed and measured at 620 nm (Sinha, 1972).

Assay of reduced glutathione (GSH)

Reduced glutathione (GSH) in gastric mucosa was assayed by the method of Ellman. At first, stomach was homogenized in phosphate buffer (0.2 M, PH-8.0). Then

0.5 mL of the tissue homogenized mixture was treated with 2.0 ml 5% TCA (Trichloroacetic acid). The mixture was kept on ice for 10-20 minutes and then centrifuged at 3000rpm for 15 minutes. After that 2.0 mL of the supernatant of homogenized mixture was treated with 1.0 ml of Ellman's reagent and 4.0 mL of 0.3 M disodium hydrogen phosphate. The absorbance of yellow colour developed was measured in a UV spectrophotometer at 412 nm within 2-3 minutes. The amount of glutathione is expressed as Unit/minute/mg-protein (Ellman, 1959).

Investigation of anti-inflammatory activity

Pharmacological investigations of ELGKMs formulation by granuloma tissue formation method through cotton pellet grafting under skin

The animals were deprived of food for 24 hours (water *ad libitum*) prior to the drug administration and received free access to water during the experiment. They were divided into five groups of six animals in each group. The first group was treated as control group receiving 1% CMC solution. Second and third groups were treated as standard groups receiving pure etodolac suspension (50, 75mg/kg b.w. respectively). Test groups were given ELGKMs (50, 75 mg/kg b.w respectively). The animals were anaesthetized with diethyl ether. Then the hair of back skin of lumber region was removed and disinfected with 70% ethanol. A subcutaneous incision was made by scalpel. A sterilized cotton pellet (60-80 mg) impregnated with 1% carrageenan solution was implanted. The wounded skin was then closed with a stainless steel suture clip. The animals were kept in separate cages. Then they were treated with pure etodolac and ELGKMs suspension per oral for 6days (through oral gavages). On the 7th day, the animals were sacrificed under anaesthetized and cotton pellets surrounded with granuloma tissue were removed. The weight of wet cotton pellets was taken and recorded. Then they were dried at 60 ºC until the weight remained constant. Finally, weights of dry cotton pellets were recorded. The net weight of cotton pellets was determined by subtracting weight of dry cotton pellets from weight of wet cotton pellets.

Haematological assay

At the end of the experimental, on 7th day the blood was collected directly from heart and used for the analysis of hematological parameters such as erythrocyte count, total and differential leukocyte count, hemoglobin concentration, platelet count and blood clotting time.

Haemoglobin estimation

The heparinized blood was taken in the Sahli Hemoglobinometer and diluted with the 0.1 N HCl until the colour matched with the standard. The data was taken from graduated cylinder and expressed as g/dl of blood.

RBC count

The blood sample was diluted 1:75 with the RBC diluting fluid using Thoma pipette after vigorous mixing, a drop of blood mixture was dropped under cover glass of Neubauer hemocytometer and corpuscles were allowed to settle for 3 minutes. The number of RBC in 80 small squares was determined under light microscope.

WBC count

The blood sample was diluted 1:20 with the WBC diluting fluid with Thoma pipette after vigorous mixing a drop of blood mixture was dropped under cover glass of Neubauer hemocytometer and corpuscles were allowed to settle for 3 minutes. The number of WBC in 16 small squares was determined under light microscope (Armour, Blood, Belden, 1965; Wintrobe *et al.*, 1976).

Differential leukocyte count and platelet count

The heparinized blood was analysed by digital automatic hematology analyser/blood cell counter (Care Well Biotech Pvt. Ltd., India) in Nilam-The Complete Care, 26B, Ahiripukur Road (Lower Range- Beck Bagan) Kolkata-700019, India and the results were obtained.

Blood clotting time

The blood sample was taken using a glass capillary from orbital plexus of the eye of each rat and the time was noted down. Small pieces of capillary were broken from one end at every 30s till fibrin threads of blood appeared between the broken ends of capillary (Ghai, 1990).

Biochemical assay

The non-heparinized blood was allowed to coagulate. Then the coagulated blood was centrifuged at 4000rpm for 20 minutes and the serum was separated. The biochemical parameters like concentration of glucose, total bilirubin (TB), aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP), Serum Calcium and Serum Iron were estimated using commercially available standard kits (Nilam, The Complete Care) on an automatic analyzer (Merck Pvt. Ltd.).

Histopathology

Stomach, Liver, Kidney and Heart of treated rats from control and treated groups were dissected into small sections and preserved in cedar wood oil. Infiltration was done by dipping the tissues in xylene: paraffin wax in 1:1 ratio at 60º C for 1 hour and then tissues were dipped in molten paraffin at 60ºC for 1 hour. The processed tissues were embedded in the molten wax for section cutting. Thin section of the paraffin blocks containing tissue was done by rotary microtome. Then the slides were stained with eosin and hematoxylin and mounted with dextrene polystyrene xylene and examined microscopically for pathological examination.

Statistical analysis

All the results were expressed as mean ± SEM. The results were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's test through the computer program Graph Pad Instat 3. P value <0.01and >0.05 were considered statistically significant whereas p value <0.05 was considered statistically not significant.

RESULT AND DISCUSSION

Gastric irritation properties of orally taken compounds were evaluated in rats kept in fasting condition (Vogel, 2002). The investigations of ulcerogenic activity of pure etodolac and etodolac loaded gum katira microspheres (ELGKM) treated rats indicated that all the ELGKMs formulation treated rats showed a remarkable decrease in ulcerogenic properties compared to pure etodolac-treated rats. The statistical analysis of ulcer index of treated rats suggested that there were significant differences among the groups (Table II). Etodolac caused gastrointestinal damages and ulcers in pure form as well as microspheres dosages form (Figure 1). But pure etodolac form (50 mg/kg b.w. and 75 mg/kg b.w. respectively) showed a significant gastric damages and ulcerations whereas the ELGKMs formulation (50 mg/kg b.w. and 75 mg/kg b.w) showed significantly lesser gastric damages and ulceration. Based on ulcerogenic study on treated rats, the results indicated that ELGKMs formulation (50 mg/kg and 75 mg/kg) possess markedly minimized the gastrointestinal damaging tendency. Biochemical analysis of stomach tissue homogenate of treated rats also supported that ELGKM decreased the gastric damages properties of etodolac in comparison to pure etodolac-treated rats. The gastric damages, ulcerations and others toxic conditions of stomach causes marked reduction in some enzyme concentration (Total protein, Catalase and GSH) and increases the concentration of TBARS (Adeyemi *et al.*, 2015). From the biochemical investigation, it was noted that the both the pure etodolac and ELGKMs formulations increased the level of TBARS and decreased the level of some enzymes concentrations in stomach tissues but the increase in level of TBARS was much less with the ELGKMs formulations than the pure drug. Similarly the decrease in enzyme concentration was less with the ELGKMs formulations in comparison to the pure drug. From the statistical analysis it was found that most of the biochemical parameters (TP, TBARS, CAT and GSH) of rat stomach tissue homogenates were significantly different at 0.05 and 0.01 level as compared to control group (Table III).

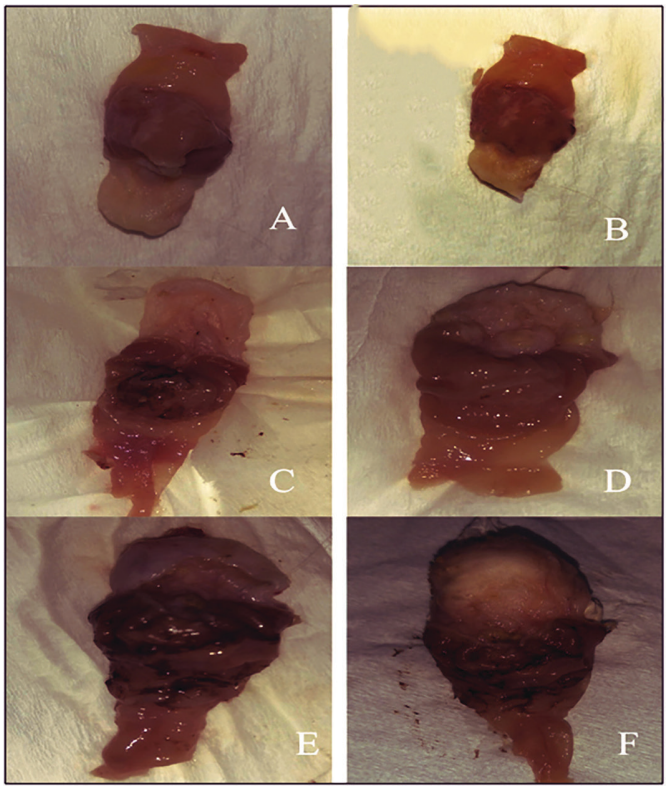

FIGURE 1 - Study of Ulcerogenic activity in Rats: **A**) Control Group; **B**) Blank Microspheres (Without etodolac); **C**) 50 mg of pure etodolac; **D**) 50mg equal quantity of Etodolac in ELGKMs; **E**) 75 mg of pure etodolac; and **F**) 75 mg equal quantity of etodolac in ELGKMs.

Granuloma tissue formation is the key characteristics in chronic inflammatory conditions. Most of the time, animal models of granuloma tissue formation are being employed for the screening of anti-inflammatory drugs (Paschapur *et al*., 2009). The cotton pellets test was first described by Meier, Schuler and

TABLE II - Comparative study of Ulcerogenic activity in treated rats

Treatment	Dose (PO, mg/kg b.w)	Total Score	Ulcer Index
Control	-	0	0
Blank Microspheres	Equal quantity on basis of 50mg Etodolac	**4.83±0.75**	**0.48**
Pure Etodolac	50mg	**17.16±1.16**	**1.71**
ELGKMs	50mg*	**10.33±1.21**	**1.03**
Pure Etodolac	75mg	**23.16±2.04**	**2.31**
ELGKMs	75mg*	**18.50±1.04**	**1.85**

*Dose equal quantity of Etodolac present in ELGKMs. Data are taken as mean ±SD, P<0.01 as compared to Blank group. Table value (df 5, 25) = 3.86 at 0.01 level. The results were analyzed by one-way ANOVA followed by Dunnett's Multiple Comparison Test. Each value represents the mean ±SEM of 6 animals.

TABLE III - Biochemical parameters of Stomach of treated rats

Parameters	Control	Blank Microspheres	Etodolac (50 mg/kg)	ELGKMs (50 mg/kg)	Etodolac (75 mg/kg)	ELGKMs (75 mg/kg)
TP (µg/ml)	24.35±0.98	22.12±0.90	13.01±1.40**	17.74±1.7**	9.02±1.03**	11.95±0.5**
TBARS (nMol/µg protein)	0.96±0.66	1.34±0.46*	1.76±0.39	1.22±0.42*	2.36±0.28**	1.86±0.16**
CAT (U/min/µg protein)	2.66±0.48	2.40±0.22*	1.60±0.34**	2.16±0.24*	0.72±0.38**	1.28±0.39**
GSH (U/min/µg protein)	2.94±0.19	2.77±0.20*	2.07±0.51**	2.32±0.10**	1.25±0.14**	1.62±0.23**

Data are taken as mean ±SD, P<0.05, P*>0.05 and P**<0.01 as compared to control group. Table value (df 5, 30) =2.53 at 0.05 level and 3.70 at 0.01 level. The results were analyzed by one-way ANOVA followed by Dunnett's Multiple Comparison Test. Each value represents the mean ±SEM of 6 animals. [P<0.05, Biochemical parameters are not significant compared to respective control group for these respective parameters]

Desuelles (1950) for the evaluation of non-steroidal anti-inflammatory drugs (Meier, Schuler, Desaulles, 1950). Granuloma tissue formation was described by Vogel that reflected the chronic proliferative inflammation (Vogel, 1970). According to Finney and Somers, the time interval between implantation of the cotton pellets and removal of granuloma tissue associated cotton pellet should be six days (Finney, Somers, 1958). In case of pure etodolac-treated rats (50, 75 mg/kg b.w), the average weights of cotton pellets were higher in comparison to ELGKMs treated rats of 50 or 75 mg/kg b.w respectively (Table IV). The probable reason behind these results was that ELGKMs delivered the drugs for a longer period of time (10-12 hours) at the inflammatory sites of treated rats. As a result, the granuloma tissue formation was reduced in ELGKMs treated groups. The graphical representation of cotton pellet test has been displayed in Figure 2**.** The results of cotton pellets test suggested that ELGKM, sustained-release formulation was the more efficacious in the treatment of chronic pain and inflammatory conditions.

WBC cells take part directly in the inflammatory process in the human body mechanisms (Kytridis, Manetas, 2006). The WBC counts such as lymphocytes are significantly increased in response to cytotoxicity (Robin, 1974). The hematological changes such as decrease in RBC count, Hemoglobin concentration, Lymphocytes, Eosinophil and increase in clotting times and platelet counts were observed in treated groups.

Paulus and Whitehouse stated that some of the complements took part a vital role in the protective mechanism of the tissue or organ against exogenous or endogenous injuries (Buer, 2014). The complements activation results in formation of many more pathogenic and inflammatory factors which cause smooth muscle contraction, increases capillary permeability, accumulation of migrated leukocytes and lysis of platelets. The following experimental results of platelet count in treated

TABLE IV - Comparative granuloma tissue weight of implanted cotton pellets in rats

Treatment	Dose (PO, mg/kg b.w)	Granuloma Tissue weight in mg ±SD
Control	-	131.5± 6.091
Pure Etodolac	50mg	112.3±6.022
ELGKMs	50mg*	102.8±6.242
Pure Etodolac	75mg	78.17±7.985
ELGKMs	75mg*	69.83±5.565

*Dose equal quantity of Etodolac present in ELGKMs. P<0.01 compared with control group. Table F value (df 4,25)= 2.76 at 0.05 level and 4.18 at 0.01 level respectively. The results were analyzed by one-way ANOVA followed by Dunnett's Multiple Comparison Test. Each value represents the mean ±SEM of 6 animals.

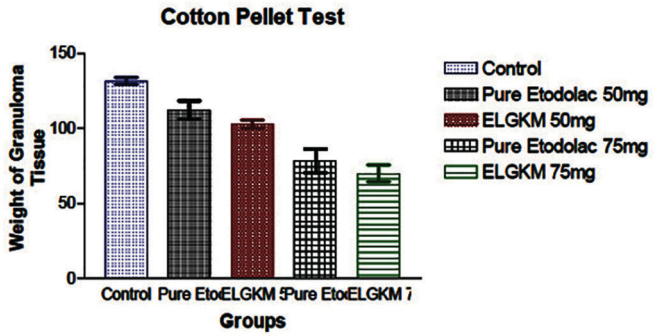

FIGURE 2 - Graphical representation of comparative Anti-inflammatory efficacy by cotton pellet implantation method in rat.

rats followed the explanation. The results of hematological analysis among the animal groups indicated that ELGKMs formulation (50 mg/kg and 75 mg/kg) treated groups suggested better results in comparison to the other animal groups. There were significant differences at 0.05 and 0.01 levels in hematological parameters among the drug treated groups as compared to control group (Table V).

Lysosomal enzymes such as ALT, AST and ALP played an important role in the initiation of inflammation, tissue injury and connective tissue breakdown (Weissmann *et al.*, 1971). Researcher Anderson found that levels of lysosomal enzymes were significantly increased in inflamed tissue or serum of rats as compare to normal rats (Anderson, 1970). According to Adam, 1998 and Crook, 2006 (Adam, 1998; Crook, 2006) stated that in toxic stages, the blood level of SGOT and SGPT were known to increase significantly. The higher concentration of SGOT, SGPT and Alkaline Phosphatase may be an indication of tissue damage in liver and kidney. The biochemical analysis of pure etodolac and ELGKM treated rats blood indicated that comparatively decrease in amount of total protein, glucose are less with ELGKM than that

TABLE V - Hematological parameters of Microspheres treated rats

Hematological Parameters	Control Group	ELGKM (50 mg/kg b.w)	ELGKM (75 mg/kg b.w.)	Pure Etodolac (75 mg/kg b.w)
Haemoglobin (gm%)	12.94 ± 1.69	10.60 ± 0.61**	9.55 ± 1.83**	11.52 ± 0.53*
R.B.C. (millions/cu.mm.)	7.77 ± 1.10	7.17 ± 0.27*	6.64 ± 0.29**	7.55 ± 0.09*
W.B.C. (cu.mm.)	14900± 904.99	13176.00 ± 50.29**	12158.33± 640.64**	14816.67± 240.14*
Lymphocyte (%)	71.64 ± 1.31	68.03 ± 0.49**	66.95 ± 0.72**	69.95 ± 0.31**
Monocyte(%)	2.69 ± 0.42	2.05 ± 0.18**	1.82 ± 0.28**	2.49 ± 0.08*
Platelet Count -(lakhs/cu.mm)	1081.17±120.43	1191.68 ±34.30	1246.33 ± 79.35**	1138.00 ± 14.69*
Cloting Time (mins)	2.313 ± 0.38	2.405 ± 0.04*	2.46 ± 0.64*	2.37 ± 0.03*

Data are taken as mean ±SD, P<0.05, P*>0.05 and P**<0.01 as compared to control group. Table value (df 3,20)=3.10 at 0.05 level and 4.94 at 0.01 level. The results were analyzed by one-way ANOVA followed by Dunnett's Multiple Comparison Test. Each value represents the mean ±SEM of 6 animals. [P<0.05, Hematological parameters are not significant compared to respective control group for these respective parameters]

of pure drug. Similarly increase in amount of SGOT SGPT and concentrations of alkaline phosphatase are more pronounce with the pure drug than that of ELGKM formulations.

Calcium is required for secretion of lysosomal enzymes from the neutrophils (Rubin, 1970; Woodwin, Wienwke, 1963). Calcium entry into the neutrophil cells provoked the accumulation of intracellular cyclic GMP which enhanced the secretion of lysosomal enzymes in the extracellular environment. Calcium levels in drug-treated rats were lower in compared to non-drug treated rats but negative results were noted on serum iron concentration. The results of biochemical parameters at different drug treated groups were analyzed by one-way ANOVA followed by Dunnett's Multiple Comparison Test. It was noted that the biochemical variables in formulation treated groups were significantly different in comparison to control group at 0.05 and 0.01 levels (Table VI).

The histological studies confirmed that significant tissue morphology was changed in etodolac treated rats (Figure 3). Pure etodolac caused more toxic environment in the rat than ELGKM formulation treated rats. The present experimental results of both pharmacological and toxicological parameters suggest that ELGKMs will be more beneficiary for the treatment of chronic pain or inflammatory conditions (Mariappan *et al*., 2011).

CONCLUSION

From the investigation of ulcerogenic activity of etodolac loaded gum Katira microspheres (ELGKMs) prepared by $W_1/O/W_2$ emulsion solvent evaporation technique showed significant decrease in the stomach ulceration in the rat's reacted with ELGKMs in comparison with the pure etodolac treated groups. The studies on anti-inflammatory activity prove beyond doubt that ELGKMs has more pronounced anti-inflammatory activity than the pure etodolac. Therefore on the basis of pharmacological evaluation of anti-inflammatory activity and ulcerogenecity of ELGKMs compared with pure etodolac, it can be said that ELGKMs showed better efficacy in comparison to pure etodolac in a chronic inflammatory condition of rats. The histological analysis of different tissues of drug treated rats indicated that a few number of damaged cells were noticed in ELGKMs treated rats than that of pure etodolac. The pharmacological and toxicological investigation of orally administered ELGKMs in rats suggested that etodolac loaded gum Katira microspheres has the potential to be developed into a better and effective sustain release formulation for the management of rheumatoid arthritis, osteoarthritis and other chronic joint pain related diseases.

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TABLE VI - Biochemical parameters of microspheres treated in rats

Biochemical Parameters	Control Group	ELGKMS (50 mg/kg b.w)	ELGKMs (75 mg/kg b.w.)	Pure Etodolac (75 mg/kg b.w.)
Total Bilirubin (mg/dl)	0.482 ± 0.019	0.457 ± 0.021*	0.433 ± 0.047*	0.467 ± 0.029*
SGOT (U/L)	195.50±3.834	292.83± 26.21	304.33± 16.98**	228.33 ± 12.91*
SGPT (U/L)	26.00 ± 3.85	39.33 ± 2.58**	40.33 ± 5.92**	33.50 ± 4.68
Blood Sugar (mg/dl)	108.67±5.125	84.17 ± 5.35**	80.00 ± 7.51**	99.00 ± 6.03
Alkaline Phosphatase (U/L)	287.00± 8.25	337.00 ± 8.94**	372.33 ± 5.05**	303.00 ± 11.10**
Serum Triglyceride (mg/dl)	72.83 ± 4.53	116.67± 11.81**	145.00 ± 6.06**	99.50 ± 14.79**
Serum Calcium (mg/dl)	9.20±2.05	8.20±0.59*	6.70±1.78	8.60±1.26*
Serum Iron (ug/dl)	98.00±3.47	143.00±6.14**	177.00±4.56**	121.00±2.45**

Data are taken as mean ±SD, P<0.05, P*>0.05 and P**<0.01 as compared to control group. Table value (df 3,20)=3.10 at 0.05 level and 4.94 at 0.01 level. The results were analyzed by one-way ANOVA followed by Dunnett's Multiple Comparison Test. Each value represents the mean ±SEM of 6 animals. [P<0.05, Biochemical parameters are not significant compared to respective control group for these respective parameters]

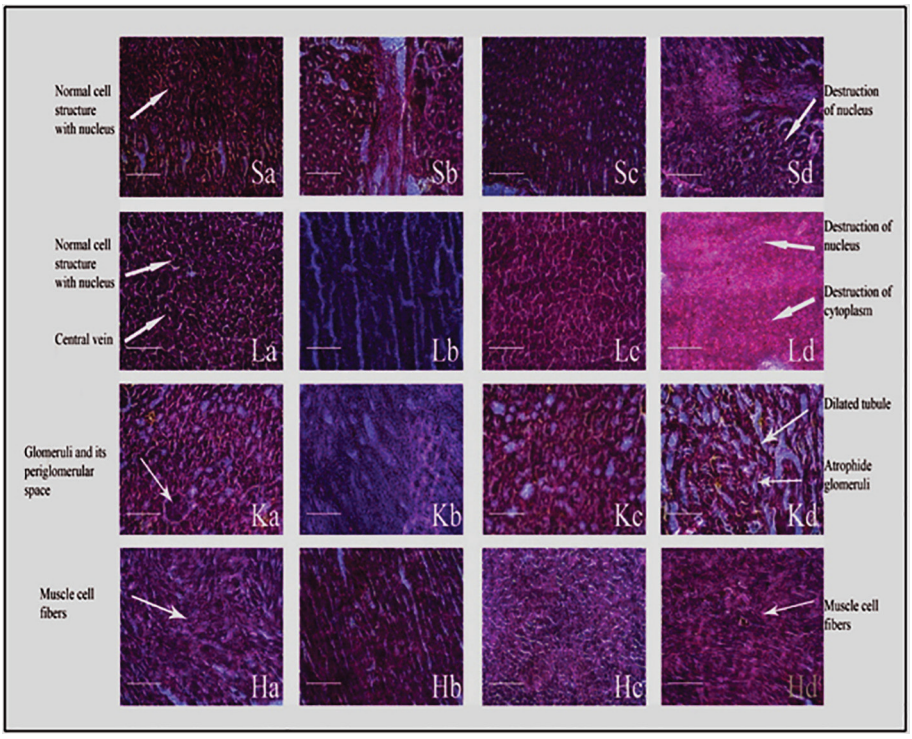

FIGURE 3 - Study of Histological changes of Stomach(**S**), Liver (**L**), Kidney (**K**) and Heart (**H**) of Rats: **Sa**, **Sb**, **Sc**, and **Sd** are Control, 75 mg of pure etodolac, 50 mg and 75 mg of equal quantity of etodolac in ELGKMs respectively; **La, Lb, Lc** and **Ld** are Control, 75 mg of pure etodolac, 50 mg and 75mg of equal quantity of etodolac in ELGKMs respectively; **Ka, Kb, Kc** and **Kd** are Control, 75 mg of pure etodolac, 50 mg and 75 mg of equal quantity of etodolac in ELGKMs respectively; **Ha, Hb, Hc** and **Hd** are Control, 75 mg of pure etodolac, 50 mg and 75 mg of the equal quantity of etodolac in ELGKMs respectively.

DECLARATION OF INTEREST

The authors state no conflict of interest.

REFERENCES

Adam SEI. Toxic effects of Francoeuriacrispa in rats. Phytother Res. 1998;12(7):476-479.

Adeyemi OT, Osilesi O, Adebawo OO, Onajobi FD, Oyedemi SO, Afolayan AJ. Alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in selected tissues of rats fed on processed atlantic horse mackerel (Trachurustrachurus). Adv Biosci Biotechnol. 2015;6(3):139-152.

Anderson AJ.Lysosomal enzyme activity in rats with adjuvant-induced arthritis. Ann Rheum Dis. 1970;29(3):307.

Armour DFE, Blood FR, Belden DA. In the manual of laboratory work in mammalian physiology. 3rd ed. Chicago: The University of Chicago, Liilious; 1965. 216 p.

Buer JK. Orginals and impact of the term 'NASID'. Inflammopharmacol. 2014; 22(5):263-267.

Craig CR,Stitzer RE. Modern pharmacology with clinical application. 6th ed. USA: Lippincott William and Wilkins; 2004. 832 p.

Crook MA. Clinical chemistry and metabolic medicine. 7th ed. London: Hodder Arnold; 2006. 426p.

Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys. 1959;82(1):70-77.

Finney RS, Somers GF.The anti-inflammatory activity of glycyrrhetinic acid and derivatives.ʃ J Pharm Pharmacol. 1958;10(1):613-620.

Ghai CL. Textbook of practical physiology. New Delhi: Jaypee Medical Publishers; 1990. 371 p.

Glaser K, Sung ML, O'Neill K, Hartman D, Carlson R, Kreft A, et al. Etodolac selectively inhibits human prostaglandin G/H synthase 2 (PGHS-2) versus human PGHS-1. Eur J Pharmacol. 1995;281(1):107-111.

Indian Pharmacopoeia Commission. Indian Pharmacopoeia.7th ed. Ghaziabad; 2014. v.2, p. 1718.

Kytridis VP, Manetas Y. Mesophyll versus epidermal anthocyanins as potential in vivo antioxidants: evidence linking the putative antioxidant role to the proximity of oxy-radical source. J Exp Bot. 2006;57(10):2203-2210.

Main IH, Whittle BJ. Investigation of the vasodilator and antisecretory role of prostaglandins in the rat gastric mucosa by use of non-steroidal anti-inflammatory drugs. Br J Pharmacol. 1975;53(2):217-24.

Mariappan G, Saha BP, Sutharson L, Singh A, Garg S, et al. Analgesic, anti-inflammatory, antipyretic and toxicological evaluation of some newer 3-methyl pyrazolone derivatives. Saudi Pharm J. 2011;19(2):115-22.

Meier R, Schuler W, Desaulles P. Zurfrage des mechanismus der hemmung des bindegewebswachstumsdurch cortisone. Experientia. 1950;6(12):469-71.

Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 1979;95(2):351-8.

Paschapur MS, Patil MB, Kumar R, Patil SR. Evaluation of anti-inflammatory activity of ethanolic extract of *Borassus flabellifer* L. male flowers (inflorescences) in experimental animals. J Med Plant Res. 2009;3(2):49-54.

Peterson GL. Review of the Folin phenol protein quantitation method of Lowry, Rosebrough, Farr and Randall. Anal Biochem. 1979;100(2):201-220.

Rainsford KD. Mechanism of gastrointestinal toxicity of nonsteroidal anti-inflammatory drugs. Scand J Gastroenterol. 1989;24(165):9-16.

Robins SL. Lymph nodes and spleen: pathologic basis of disease. Philadelphia: WB Saunders Co; 1974. 1050 p.
Rubin RP. The role of calcium in the release of neurotransmitter substances and hormones. Pharmacol Rev. 1970;22(3):389-428.

Ruhidas B, Naskar D, Banerjee S, Karan S, Chatterjee TK. Evaluation of gum katira as a model sustained release adjuvant in the preparation of etodolac loaded microsphere. Indian J Pharm Educ. 2016;50(1):1-13.

Shojaei AH. Buccal mucosa as a route for systemic drug delivery: a review. J Pharm Pharm Sci. 1998;1(1):15-30.

Sinha AK. Colorimetric assay of catalase. Anal Biochem. 1972;47(2):389-94.

Tammara VK, Narurkar MM, Crider AM, Khan MA. Synthesis and evaluation of morpholinoalkyl ester prodrugs of indomethacin and naproxen. Pharm Res. 1993;10(8):1191-9.

Vogel HG, editor. Drug discovery and evaluation: pharmacological assays. 2nd ed. New York: Springer-Verlag; Berlin: Heidelberg; 2002;1408.

Vogel HG. Das Glasstabgranulom, eine Methode zur Untersuchung der Wirkung von Corticosteroiden auf Gewicht, Festigkeit und chemische Zusammensetzung des Granulationsgewebes an Ratten. Arzneim Forsch Drug Res. 1970;20:1911-8.

Vyas S, Trivedi P, Chaturvedi SC. Dextran-etodolac conjugates: synthesis, in vitro and in vivo evaluation. Acta Pol Pharm. 2009;66(2):201-6.

Weissmann G, Zurier RB, Spieler PJ, Goldstein IM. Mechanisms of lysosomal enzyme release from leukocytes exposed to immune complexes and other particles. J Exp Med. 1971;134(3):149-65.

Wintrobe MM, Lee GR, Boggs DR, Bithel TC, Anthens JW, Foerersters J. Clinical Hematology. 7th ed. Philadelphia: Lea & Febiger; 1976.1896 p.

Woodin AM, Wieneke AA. The accumulation of calcium by the polymorphonuclear leucocyte treated with staphylococcal leucocidin and its significance in the extrusion of protein. Biochem J.1963;87(3):487.

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