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ANTI- INFLAMMATORY, ANALGESIC & ULCEROGENIC ACTIVITY OF KETOPROFEN GLUCOPYRANOSIDE CONJUGATES

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ABSTRACT

Ketoprofen is a non-steroidal anti-inflammatory drug. Glucopyranoside Conjugates of Ketoprofen have been synthesized and evaluated for anti- inflammatory, analgesic and ulcerogenic activities. *In vivo* biological screening in mice and rats indicated that conjugates improved analgesic and anti-inflammatory activities with significantly reduced ulcerogenicity compared to the parent drug.

INTRODUCTION: Ketoprofen is a potent anti-inflammatory drug. Chemically, it is propionic acid derivative provided as a recemic mixture. This non-steroidal and non-narcotic drug is administered systemically (*via* oral and parenteral route) for the control of mild to moderate pain as well as of some postoperative and cancer pain [1]. It has similar pharmacological actions to other drugs in this class such as ibuprofen, fenoprofen and naproxen.

It inhibits the synthesis of prostaglandin (PG) by inhibiting the enzyme cyclooxygenase (COX). Administration of this non-selective COX inhibitor by oral route causes many gastrointestinal side effects, like nausea, vomiting, gastric irritation, peptic ulceration and bleeding that limit its clinical use [2]. PGs are ubiquitous compounds that mediate a variety of physiologic and pathologic processes. Under normal physiologic conditions, PGs play an essential homeostatic role in cytoprotection of gastric mucosa, hemostasis, renal function, gestation and parturition [3- 5].

It is a well accepted fact that gastrointestinal lesions produced by NSAIDs are due to two different mechanisms: (a) direct contact with gastric mucosa through oral dose and (b) systemic effect which may be manifested by after intravenous dosing[6]. Masking the acidic group of NSAIDs with a view to decrease the gastrointestinal toxicity due to direct injury has been postulated[7- 10]. The purpose of this study was to mask the free acidic group by synthesizing its glucopyranoside derivative and evaluate its anti-inflammatory activity, analgesic activity and gastrointestinal toxicity.

EXPERIMENTAL:

Chemistry: Melting points were determined by Superfit Melting Point Determination Apparatus in open capillary tube and were uncorrected (Table 1). IR spectra were taken on Nujol mulls between salt plates. ^{1}H-NMR spectra were recorded, in $CDCl_3$ solution, on a Bruker Avance II 400 spectrometer. The chemical shift are reported in part per million (δ, ppm) downfield from tetramethylsilane, which was used as internal standard. Mass spectra were also recorded in $CDCl_3$ solution.

Synthetic procedure (Scheme 1):

Synthesis of 2, 3, 4, 6- tetra- o- acetyl α- D-Glucopyranosyl Bromide (3): Acetic anhydride (400mL) was placed in a two-necked flask kept in an ice-salt bath on a magnetic stirrer. The temperature was maintained at 4°C±1°C and 2.4 mL of 60% perchloric acid is added drop wise. The reaction mixture was allowed to reach room temperature gradually. α- D- Glucose (2) (100 g) as dry powder was added in portions, with continuous stirring, the temperature being maintained at 30 – 40 °C. Red Phosphorus (31g) was added immediately followed by bromine (58mL) drop wise, keeping the temperature maintained at 20°C. Water (36mL) was added over a period of half an hour, stirring and cooling being continued and the temperature being maintained below 20°C. The reaction mixture was allowed to stand for 2 hours at room temperature, and then dichloromethane (300mL) was added and then filtered. The filtrate was washed twice with iced water. The dichloromethane layer was run in saturated solution of $NaHCO_3$ to which was added some crushed ice. After the vigorous

evolution of CO_2, the dichloromethane layer was run into a flask containing 10g of powdered activated silica gel and filtered after 10min; the solvent was removed by the use of rotary evaporator. The syrupy mass crystallized as a thick layer around the inside of the flask. Portions of the solid was transferred to a pestle mortar and ground with a 2:1 mixture of light petroleum (B.P. 60-80°C) & dry ether. The combined slurry was filtered and washed with a light-petroleum ether solvent mixture and then with 50mL of previously chilled (0°C) dry ether.

Synthesis of Ketoprofen tetraacetyl ß- D-Glucopyranosyl derivative (KTAG/ 4): To a solution of Ketoprofen (0.61 g, 3 mmol) and 2, 3, 4, 6- tetra- o- acetyl α- D-glucopyranosyl bromide (1.64 g, 4 mmol) in dichloromethane was added Tetrabutyl ammonium bromide (0.644 g, 2 mmol) with stirring at 5°C. Aqueous sodium hydroxide (10%,10 mL) was added to it drop wise over a period of 30 min and the reaction mixture further stirred for 24 hr. The organic layer separated out, was washed with water followed by 5% aqueous $NaHCO_3$ solution, again with water. The product was dried and concentrated in vacuo. A semi-solid mass so obtained was purified on a column of silica gel and crystallized from ethanol as colourless needles. Yield was 81.5%.

IR (KBr): 2961.6 and 2874.0, 3060.7, 1651.6, 1594.8, 1461.8 and 1381.0 cm^{-1}

NMR ($CDCl_3$): 2.01(s, 12H, $OCOCH_3$), 7.31-7.70(m, 9H, aromatic), 3.78(m, 1H, CH), 1.52(m, 3H, CH_3), 4.09(s, 2H, CH_2)

Mass: 584.4 (M^+)

Synthesis of Ketoprofen ß- D-Glucopyranosyl derivative (KG/ 5): The tetra acetyl derivative was deacetylated by adding 1.45 mL of 0.5% sodium methoxide solution and kept at room temperature for 45 min. The reaction mixture was neutralized with Amberlite IR 120, SD fine, filtered and concentrated in vacuo. A semi-solid mass so obtained was crystallized from absolute ethanol as colourless compound. Yield was 67%.

IR (KBr): 3405.8, 2961.6 and 2874.0, 1742.2, 1461.8, 1200.9 cm^{-1}.

NMR ($CDCl_3$): 2.0(s, 4H, OH), 7.31-7.70(m, 9H, aromatic), 3.78(m, 1H, CH), 1.52(m, 3H, CH_3), 3.79(m,2H,CH_2)

Mass: 416.1 (M^+)

Biological Evaluation:

Anti- inflammatory activity: Anti-inflammatory activity was determined by Carrageenan induced rat paw edema method. Six male Sprague-Dawley rats with a body weight between 100 and 150 g were used for each test and control groups. Just before injection of the test compounds the volume of the paw was measured plethysmometrically[11]. Animals were pretreated intraperitoneally with 1mL of 20mL/kg of either test compounds or ketoprofen drug. The control group received the same volume of the vehicle. Edema was induced after one hour by sub planter injection of 0.05 mL of a 1% solution of carrageenan into the left hind paw. The increase in paw volume was determined 2, 4, 6, 8 and 24 h. The percentage anti-inflammatory activity was calculated by the formula: anti-inflammatory activity= (1-dt/dc)/100 where dt is the difference of paw volume in drug/test compounds treated groups and

dc is the difference in paw volume of the control group.

Analgesic activity: Analgesic activity was determined in mice by Tail flick method. The pre-screened animals (reaction time: 3-4 sec) were divided into control, standard & test groups. Ketoprofen 20 mg/kg acted as the standard drug. The drugs were administered intraperitoneally. The tail flick latency was assessed by the analgesiometer (Inco, India). The strength of the current passing through the naked nicrome wire was kept constant at 6 Amps. The distance between the heat source and the tail skin was 1.5 cm. The site of application of the radiant heat in the tail was maintained at 2.5 cm, measured from the root of the tail. The cut-off reaction time was fixed at 10 sec to avoid tissue damage[12].

Ulcerogenic activity: The Albino rats of either sex (100-150g) were fasted for 12 hours. They're administered orally an aqueous suspension of the drug twice a day over a two-day period. The rats were then sacrificed the day after giving the final dose. To determine the gastric mucosal damage, rat stomachs were removed, opened along the length of greater curvature and cleaned of the debris, washed and examined under a microscope (10 x) & the ulcers were scored as;

0.0	Normal colour stomach
0.5	Red coloration
1.0	Spot ulcers
1.5	Hemorrhagic streaks
2.0	Ulcers > 3mm but < 5mm
3.0	Ulcers > 5mm

The mean ulcer score for each animal was expressed as Ulcer/Lesion Index (LI). For assessing the incidence of ulceration, the rats showing ulcers greater than 0.5mm in gastric mucosa were considered to have a positive ulcerogenic response[13].

RESULTS & DISCUSSION: The glucopyranoside derivatives were synthesized successfully and evaluated for their analgesic, anti-inflammatory and gastrointestinal toxicity. They showed improved anti-inflammatory and analgesic activity over the parent drug. The test compound **5** showed 64.28 % inhibition in edema as compared to 59.52 % and 52.38 % inhibition by **4** and **1** respectively in the case of anti-inflammatory activity. In the tail flick method, reaction time increased significantly for the test and standard groups when compared to the predrug reaction time. The test drug produced a dose dependent increase in the reaction time at various time intervals of observation. In comparison to ketoprofen, the test compound **5** was found to be considerably less ulcerogenic indicating that GI toxicity due to direct contact of the carboxylic group has been reduced. The results are mentioned in Photograph 1 and Table 1.

Table1: Comparative Chart of Prepared comps 5, 4 & Ketoprofen 1 With respect to their m.p. and biological properties

Compound	Ketoprofen	4	5
Melting Point	96^0C	$112\text{-}114^0$C	$163\text{-}164^0$C
Anti-inflammatory activity (%)	52.38	59.52	64.28
Analgesic activity (%)	45.67	71.87	81.73
% of Animals with ulcer	100	66.66	33.33
Ulcer Index	6.0	4.0	1.5

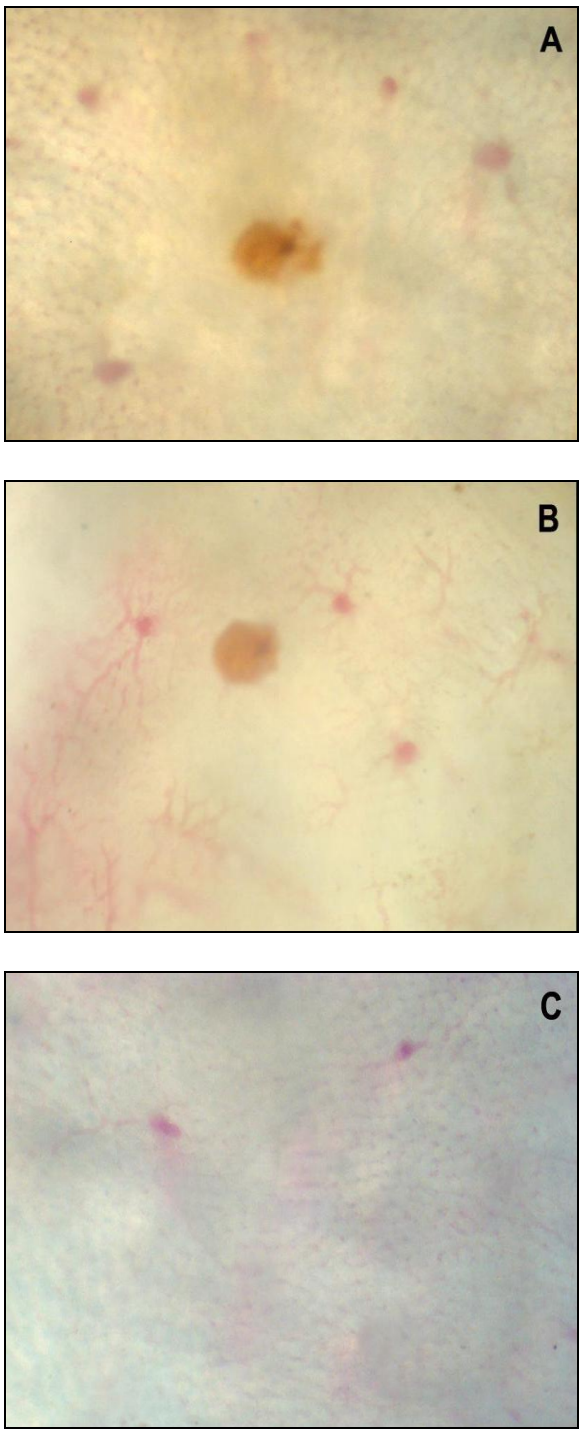

Fig. 1: (A) Ulcers induced by Ketoprofen (pure drug); (B) Ulcers induced by KTAG (intermediate conjugate) and (C) Ulcers induced by KG (final conjugate)

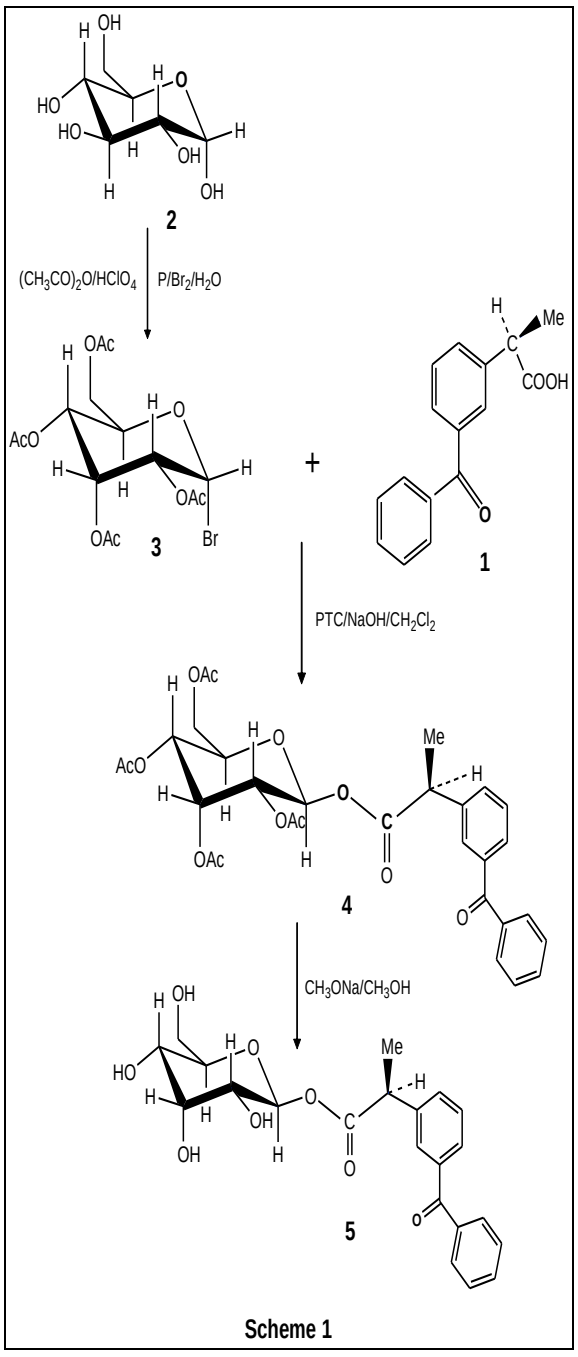

Fig. 2: Synthesis of glucopyranoside derivatives

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REFERENCES:

1. Brocks DR and Jamali F. Pharmacokinetics of ketorolac tromethamine, Clin. Pharmacokin. 1992: 415– 427.
2. Wagner W, Khanna P and Furst DE: Nonsteroidal Anti-inflammatory Drugs, Disease-modifying Antirheumatic Drugs, Nonopioid Analgesics and Drugs Used in Gout, in Basic and Clinical Pharmacology (Ed. B. G. Katzung), 9th ed., McGraw-Hill, New York 2004; 586.
3. Vane JR, Bakhle YS, Botting RM. Ann. Rev. Pharmacol. Toxicol. 1998; 38: 97-120.
4. Narumiya S, FitzGerald GA. J. Clin. Invest. 2001; 108: 25-30.
5. Smith WL, Marnett LJ, DeWitt DL. Pharmacol. Ther. 1991; 49:153-179.
6. Jones G. In Design of Prodrugs, edited by H Bundgaard (Elsevier-Amstr.), 1989;11
7. Bundgaard H & Nielsen NM. Int J. Pharm. 1988; 43:101.
8. Rainsford KD& Whitehouse MN: J Pharm Pharmacol. 1976; 28: 599.
9. Whitehouse W & Rainsford KD. J Pharm Pharmacol. 1980; 32:795.
10. Cioli V, Putzolu S, Rossi V & Corradino C.Toxicol Appl Pharmacol. 1980; 54: 332.
11. Chaves F, Barboza M ,Guttérrez JM. Pharmacological study of edema induced by venom of the snake Botherops Asper (Terciopelo) in mice. Toxicon. 1995; 33:31-39.
12. Sheth UK , Dadkar NK, Kamt UG. Drugs acting on CNS, Selected topics in experimental pharmacology. 1st Ed., Mohanlal B. Kothari Book Depot, Bombay 1972;36
13. Caprariis PD, Palagians F, Montenegrof M, D'amico M, Ross F. J. Pharm. Sci. 1994; 83, 1579-1581