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ORIGINAL RESEARCH

In-vivo analgesic and anti-inflammatory evaluation of synthesised novel new chromane and its analogues

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ABSTRACT

The current research protocol was carried to evaluate biological activity of synthesised new chromane and itsanalogues. New chromane {3,5,7-trihydroxy2-(4-hydroxy benzyl) chroman-4-one} isolated from dried leaves of *Dillenia indica* Linn, family Dilleniaceae is structurally relating with various reported chroman-4-one derivatives displaying remarkable *in-vivo* analgesic and anti-inflammatory activity.But the literature reveals that 0.8 – 1.0% yield of pure new chromane was obtained in isolation. Followingreported literature data of synthesis and *in-silico* study (COX2 binding);synthesized new chromane and its derivatives (SI-SX) were investigated for *in-vivo*analgesic and anti-inflammatory examination respectively.*in-vivo* analgesic study (Tail immersion and hot plate method) also displayed the significant analgesic potential of new chromane and its O-alkyl substituents (especially SI) while other synthesized compounds (SV-SX) reported for moderate to mild effects w.r.t. reference drug.Moreover, synthetic new chromane and O-alkyl substituent (SI) exhibited maximum anti-inflammatory activity also in terms of increment of paw volume and percentage inhibition of paw edema while others (SV-SX) showed mild anti-inflammatory action in comparison to referencedrug.

Keywords: New chromane, Analgesic, Anti-inflammatory, COX2, Tail immersion, Hot Plate

INTRODUCTION

Algesia (Gk. Word algesis meaning pain), either acute or chronic, is a natural defensive body mechanism produced as a result of any intrinsic or extrinsic injury which signals neurons in CNS to generate a feeling of strong distress or sensation[1].Analgesics (pain killers) including both centrally acting (morphine, thebaine) as well as peripherally acting (particularly NSAIDs like paracetamol, ibuprofen, diclofenac etc.) can be effectively utilized for management of algesia[2-3].Pharmacological inhibition of an enzyme i.e., cyclooxygenase (COX), responsible for the production of important biological mediator's

prostanoids i.e., prostaglandins (PGs), prostacyclin's and thromboxane can provide relief from pain, inflammation[4].However, suppression of COX may lead to undesired side effects such as gastric irritation and renal side effects[5-6].

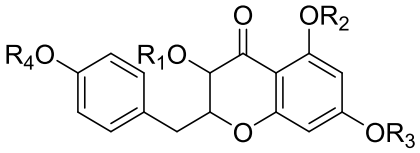
A diverse range of isolated homisoflavonones, dihydroflavonols such as hesperitin, dihydromyricetin and chroman-4-one containing compounds stilbin and silybin are reported with notable analgesic as well as anti-inflammatory activity which plays an exclusive role in framing of new anti-inflammatory drugs. [7-9]

A novel compound of chroman-4-one family, named new chromane (2-(4-hydroxybenzyl) 3,5,7-trihydroxy chroman-4-one)isolated from dried leaves of *Dillenia indica* Linn, family Dilleniaceae[10].But the literature reveals that 0.8 – 1.0% yield of pure new chromane was obtained when isolated naturally and as the literature indicates chroman-4-one nucleus has excellent potency as analgesic and anti-inflammatory[7].Therefore, new chromane and its O-substituted derivatives were synthesized at laboratory scale[11]. Furthermore, using reported literature data of synthesis and *in-silico* study (COX2 binding) [11], synthesized new chromane and its derivatives (SI-SX) were investigated for *in-vivo*analgesic and anti-inflammatoryexamination respectively.

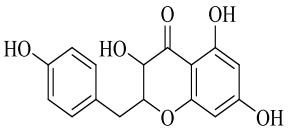
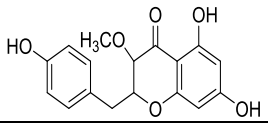
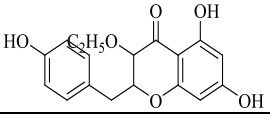
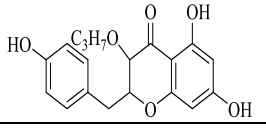
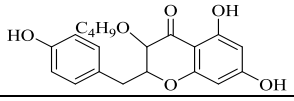
MATERIAL AND METHOD

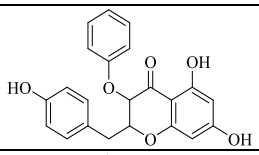
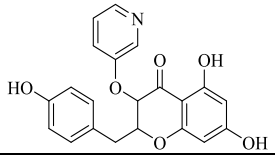
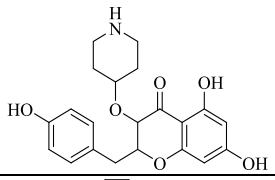
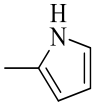
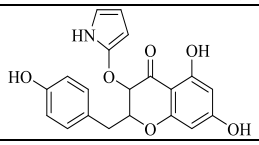
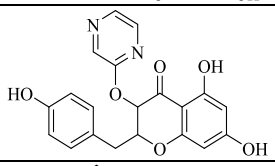
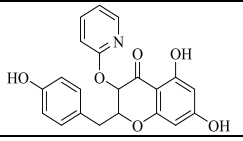
Synthesised new chromane and its analogues (**Table 1**) were investigated for oral acutetoxicity, *in-vivo* analgesic and anti-inflammatory activity[3].

Table 1: **Structural characterization of new chromane and its analogues**



Parent structure

Compounds	R_2 (C_5)	R_3 (C_7)	R_4 (C_4')	R_1 (C_3)	Structure
New Chromane	H	H	H	H	
SI	H	H	H	CH_3	
SII	H	H	H	C_2H_5	
SIII	H	H	H	C_3H_7	
SIV	H	H	H	C_4H_9	

SV	H	H	H		
SVI	H	H	H		
SVII	H	H	H		
SVIII	H	H	H		
SIX	H	H	H		
SX	H	H	H		

EXPERIMENTAL ANIMALS

Experimental protocol (MMCP-IAEC-87) was permitted by IAECInstitutional Animals Ethics Committee (1355/PO/Re/S/10/CPCSEA). Male Wistar adult rats having 180-190 g were used and kept in standard environmental conditions (temperature and standard day cycle of 23 ± 2˚C and 12 h respectively) as per prescribed CPCSEA guidelines as stated by Ministry of Environment and Forest, Government of India. A standard rodent feed and water were given to animals. Experimental animals were arranged into various groups including six rats each (**Table** 2). Animals used for toxicity studies were used further for evaluation of analgesic and anti-inflammatory activity after washout period of two weeks.

ACUTE ORAL TOXICITY

Male Wistar adult ratswere subjected to acute oral toxicity studies as per OECD 423 guidelines[12]. The animals were fasted overnight and divided into 12 groups with 6 animals in each group. Synthesised compounds i.e.,new chromane and SI-SX were administered orally at dose level of 5, 50 and 300 mg/kg body weight. The rats were observed continuously for behavioural and autonomic profiles for 2 hrs and for any signs of toxicity or mortality up to 72 hr. After 7-14 days washout period, safe animals were used for *in-vivo* biological evaluation e.g., analgesic and anti-inflammatory study.

ANALGESIC ACTIVITY

Hot plate and Tail immersionmethods were employed for the estimation of *in-vivo* analgesic activity (**Table 2**).

HOT PLATE METHOD

Male Wistar adult ratswere screened by placing them on hot plate maintained at 55 ± 1 °C and the reaction time was recorded in seconds. The time for paw licking or jumping on the hot plate was considered as a reaction time. The responses were recorded before and after 30, 60 and 120min after the administration of synthesised new chromane(100mg/kg), SI- SX (100mg/kg) and Pentazocine(50mg/kg). A cut-off time of 10 sec was used to avoid injury to the animals[14].

TAIL IMMERSION METHOD

Groups of rats were separately subjected to heat by placing 5 cm of the tail in a 500 ml beaker containing 450 ml water maintained at 55 ± 2°C before and after treatment with control, new chromane, SI-SX (test compounds, 100 mg/kg, p.o.) and pentazocine (50 mg/kg) as standard drug. The tail withdrawal from heat (flicking response) was taken as end point. A cut off time 10 sec was observed to prevent damage to the tail. Three basal reaction times for each rat at a gap of 5 min were taken to confirm normal behaviour of the rat. The reaction time at 30 min, 1 h and 2 h were recorded after the treatment and compared with standard[15].

ANTI-INFLAMMATORY STUDY

Carrageenan induced paw edema methodwas employed for the estimation of *in-vivo* anti-inflammatory activity (**Table 2**).

CARRAGEENAN INDUCED PAW EDEMA

A mark was made on both hind paws of each rat at the level ofthe lateral malleolus, so that every time the paw could be immersed in solution up to the fixed mark to ensure accurate reading of the paw volume. The initial paw volume of each rat was noted using volume displacement method before and after treatment with control, new chromane, SI-SX (test compounds, 100 mg/kg p.o.) and diclofenac sod. (Standard, 100 mg/kg). After one hour, 0.1 ml of 1 % carrageenan solution in normal saline wasinjected in the plantar region of the left hind paw of each rat.Paw volume was measured by means of volume displacement method using a plethysmometer after the carrageenan injection at 1, 2 and 3 h intervals[16].

Table 2: **Grouping of Experimental animals**

Acute oral toxicity			**Animals used for toxicity evaluation of synthesized new chromane and analogues**
Groups	**Analgesic Activity**		**Anti-inflammatory study**
	Hot plate method(mg/kg, p.o.)	Tail immersion method(mg/kg, p.o.)	Carrageenan induced paw edema(mg/kg, p.o.)
1	Control	Control	Control
2	Pentazocine	Pentazocine	Diclofenac sodium
3	New chromane	New chromane	New chromane
4	SI	SI	SI
5	SII	SII	SII
6	SIII	SIII	SIII
7	SIV	SIV	SIV
8	SV	SV	SV
9	SVI	SVI	SVI
10	SVII	SVII	SVII

11	SVIII	SVIII	SVIII
12	SIX	SIX	SIX
13	SX	SX	SX

STATISTICAL ANALYSIS

Observations formulated as mean ± SEM and results of every test sample in respective activity were corelated with respective standard drug and control by the statistic software named Sigma Stat following one-way analysis of variance (ANOVA).[16]

RESULTS AND DISCUSSION

ACUTE ORAL TOXICITY

This study was accomplished following OECD guidelines (Testing of Chemical Number 423). For this protocol, healthy & adult wistar rats (MMCP-IAEC-87) were used. Divide all animals in group of six, after fasting of 24 hours. The test compounds were given orally(b.w.), in dosages of 5, 50 and 300 mg/kg whereas control received aqueous vehicle. The testing animals were screened for next 72 hours from the period of administration of test doses, produce negligible ratio (only 2-3 animals die out of total) of mortality (LD_{50}) and also observed for any other unsui**Table** sign for next 7 days. But animals are safe and soon, tenth of the LD_{50}(also used reported ADMET lab 2.0 values)[11], that is, 100 mg/kg (ED_{50}), selected as a dose of test compounds for an analgesicand anti-inflammatory (after 7-14 days washout period).

TAIL IMMERSION METHOD

The new chromane and its various derivatives viz., SI – SX were investigated for analgesic profile in experimental rats with the help of tail immersion method with standard analgesic compound named pentazocine. **Table 3** shows the action of various synthetic compounds on mean reaction time and % maximum possible effect (MPE) of experimental animals after administration of 100 mg/kg, p.o. dose of all compounds separately at time variation such as 30, 60 and 120 min.

It is evident from **Table 3** and **Figure1** thatnew chromane and its various derivatives viz., SI – SX exhibited significant analgesic action in comparison to control and standard analgesic drug. Amongst various synthetic compounds, SI compound exhibited maximum analgesic activity in terms of significantly increased tail flicking response at 100 mg/kg followed by new chromane parent compound, SIII, SII, SIV and SV at the same dose during 2 h of the study. The compound SI and new chromane showed higher analgesic effects than pentazocine drug. Moreover, animals treated with SVI-SX showed mild analgesic action at 100 mg/kg, p.o. dose during 2 h of the study in comparison to control as well as standard drug.

Table 3: Analgesic activity of test compounds (new chromane and SI-SX) vs Pentazocine (Standard) by tail immersion method

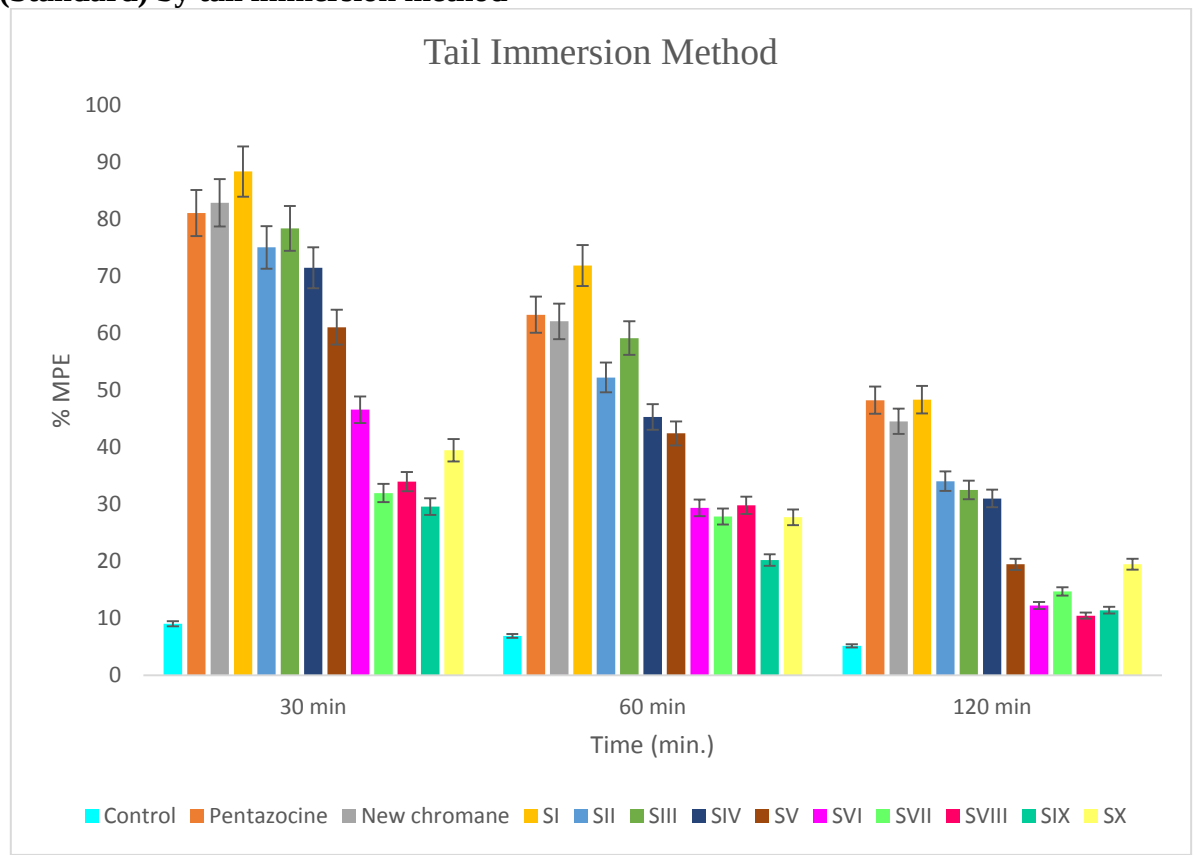
%MPE = (Actual time – Basal time)/ (Cut off time- Basal time)

Treatment	Dose (mg/kg)	Basal time (sec)	Actual time (sec)			Percentage maximum possible effect (%MPE)		
			30 min	60 min	120 min	30 min	60 min	120 min
Control	Vehicle	3.04 ± 0.13	3.67 ± 0.21[a]	3.52 ± 0.19[a]	3.40 ± 0.13[a]	9.04	6.90	5.17
Pentazocine	50	2.99 ± 0.46	8.67 ± 1.03[*a]	7.45 ± 0.89[*a]	6.39 ± 0.52[*a]	81.03	63.20	48.23

New chromane	100	3.03 ± 0.26	8.79 ± 1.05*	7.36 ± 0.82*	6.11 ± 1.33*	82.83	62.04	44.52
SI	100	2.90 ± 0.32	9.19 ± 0.75^{*b}	8.01 ± 1.10^{*b}	6.06 ± 1.03*	88.30	71.83	48.31
SII	100	2.68 ± 0.23	8.19 ± 0.75*	6.5 ± 0.84*	5.17 ± 0.75*	75.00	52.19	34.02
SIII	100	3.08 ± 0.20	8.49 ± 1.05*	7.2 ± 0.98*	5.33 ± 0.82*	78.32	59.1	32.51
SIV	100	3.00 ± 0.14	7.98 ± 0.89*	6.15 ± 0.75*	5.14 ± 0.75*	71.43	45.29	31.00
SV	100	2.62 ± 0.12	7.14 ± 1.05^{*ab}	5.73 ±0.75^{*ab}	4.06 ± 0.41^{*ab}	61.02	42.4	19.45
SVI	100	2.97 ± 0.33	6.22 ± 1.03^{*ab}	5.01 ± 0.82^{*ab}	3.84 ± 0.75^{ab}	46.57	29.34	12.23
SVII	100	2.98 ± 0.15	5.22 ± 0.75^{*ab}	4.9 ± 0.82^{ab}	3.99 ± 0.41^{ab}	31.95	27.83	14.71
SVIII	100	2.93 ± 0.08	5.33 ± 1.03^{*ab}	5.00 ± 1.03^{*ab}	3.70 ± 0.52^{ab}	33.94	29.80	10.46
SIX	100	2.95 ± 0.19	5.04 ± 1.03^{*ab}	4.41 ± 0.82^{*ab}	3.80 ± 0.75^{ab}	29.57	20.21	11.42
SX	100	2.85 ± 0.11	5.68 ± 0.52^{*ab}	4.80 ± 0.75^{*ab}	4.25 ± 0.75^{*ab}	39.45	27.70	19.48

n = 5; Mean ± S.D.; *P<0.05 vs. control; aP<0.05 vs. standard; bP<0.05 vs. parent compound

Figure1: Analgesic activity of new chromane, SI-SX(test compounds) vs Pentazocine (Standard) by tail immersion method



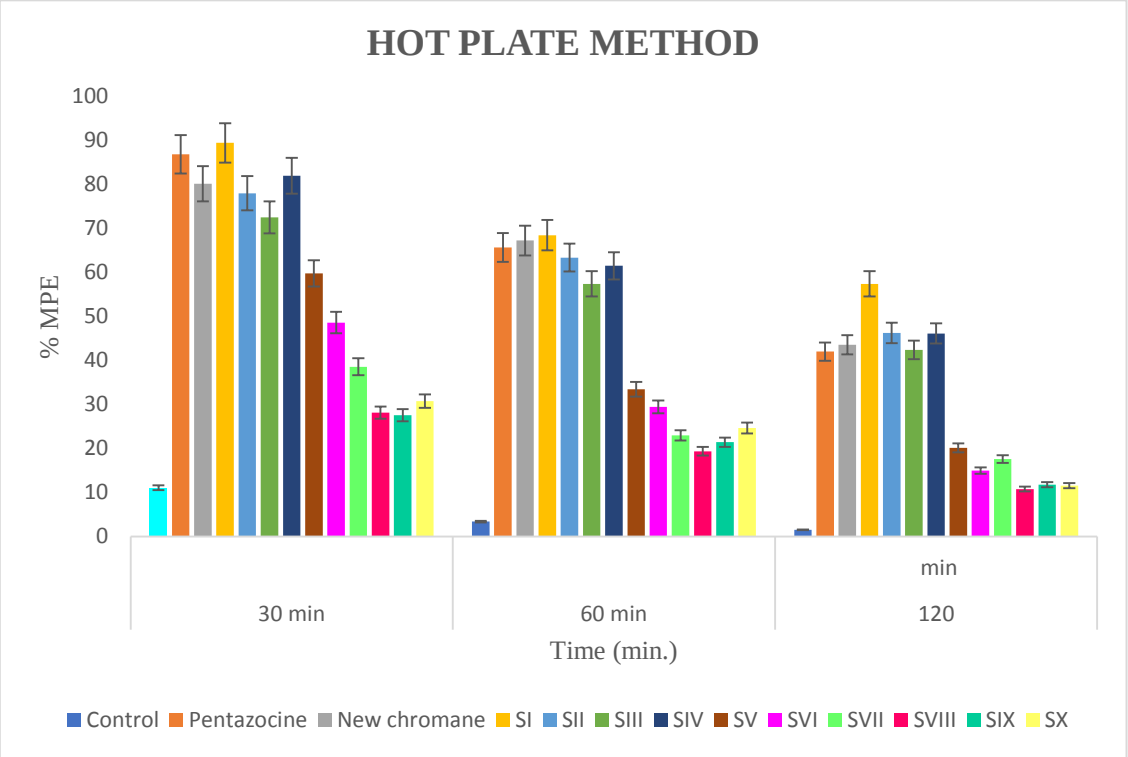
HOT PLATE METHOD

The test compounds (new chromane, SI – SX) were investigated for analgesic profile in experimental rats with the help of hot plate method using pentazocine as standard(50 mg/kg, *i.p.*)[282]. The action of various synthetic compounds on mean reaction time and % maximum possible effect (MPE) of experimental animals after administration of 100 mg/kg, (p.o.) dose of all compounds separately at time variation such as 30, 60 and 120 min is given in **Table 4**. It is evident from **Table4** and **Figure2**,new chromane synthetic parent compound and its various derivatives viz., SI – SX exhibited significant analgesic action in comparison to control and standard analgesic drug. Amongst various synthetic compounds, only SI compound exhibited maximum analgesic activity comparable to standard drug pentazocine in terms of significantly increased flicking response time at 100 mg/kg followed by new chromane at the same dose during 2 h of the study. Moreover, animals treated with SV-SX showed mild analgesic action at 100 mg/kg, p.o. during 2 h of study in comparison to control as well as standard drug.

Table 4: Analgesic activity of test compounds (new chromane and SI-SX) vs Pentazocine (standard) and by hot plate method

Treatment	Dose (mg/kg)	Basal time (sec)	Actual time (se)			%MPE		
			30 min	60 min	120 min	30 min	60 min	120 min
Control	Vehicle	3.50 ± 0.55	4.22 ± 0.63	3.72 ± 0.40[a]	3.6 ± 0.46	11.08	3.38	1.54
Pentazocine	50	3.67 ± 0.82	9.17 ± 1.33[*]	7.83 ± 0.76[*]	6.33 ± 1.03[*]	86.88	65.71	42.02
New chromane	100	3.17 ± 0.75	8.65 ± 0.52[*]	7.77 ± 0.75[*]	6.147 ± 0.98[*]	80.19	67.28	43.59
SI	100	3.33 ± 0.82	9.3 ± 0.84[*]	7.91 ± 0.54[*]	7.16± 0.97[*]	89.50	68.51	57.42
SII	100	3.17 ± 0.75	8.50 ± 0.83[*]	7.5± 0.84[*]	6.351 ± 0.81[*]	78.04	63.40	46.27
SIII	100	3.33 ± 0.81	8.149 ±1.17[*]	7.16 ± 0.75[*]	6.195 ± 0.75[*]	72.56	57.42	42.43
SIV	100	3.50 ± 0.55	8.83± 0.75[*]	7.50 ± 0.83[*]	6.5± 1.04[*]	82.00	61.54	46.15
SV	100	3.83 ± 0.75	7.51± 1.03[*ab]	5.88 ± 0.52[*ab]	5.1 ± 0.81[*ab]	59.82	33.45	20.13
SVI	100	3.17 ± 0.76	6.51 ± 0.81[*ab]	5.19 ± 0.54[*ab]	4.15 ± 0.54[*ab]	48.63	29.47	14.98
SVII	100	3.00 ± 0.63	5.72 ± 0.63[*ab]	4.61 ± 0.51[*ab]	4.198 ± 0.50[*ab]	38.57	23.00	17.57
SVIII	100	3.50 ± 0.56	5.33 ± 0.82[*ab]	4.75 ± 0.83[*ab]	4.16 ± 0.57[*ab]	28.15	19.38	10.77
SIX	100	3.33 ± 0.51	5.17 ± 1.17[*ab]	4.75 ± 0.75[*ab]	4.10 ± 0.51[*ab]	27.58	21.44	11.80
SX	100	3.50 ± 0.83	5.50 ± 0.54[*ab]	5.1 ± 0.54[*ab]	4.244 ± 0.56[*ab]	30.76	24.69	11.53

Figure2: Analgesic activity of new chromane, SI-SX(test compounds) vs Pentazocine (Standard) by hot plate method



n = 5; Mean ± S.D.; *P<0.05 vs. control; aP<0.05 vs. standard; bP<0.05 vs. parent compound

ANTI-INFLAMMATORY ACTIVITY

The new chromane synthetic parent compound and its various derivatives viz., SI – SX were investigated for anti-inflammatory profile in experimental rats with the help of paw oedema model induced by carrageenan.[283] The observations were compared with standard analgesic drug named diclofenac sodium (100 mg/kg, *i.p.*).**Table 5** suggesting the action of various synthetic compounds on increment of paw volume of the experimental animals after administartion of 100 mg/kg, p.o. dose of all compounds separately at different time intervals such as 1, 2 and 3 h.It is evident from **Table5** and **Figure3**test compounds exhibit significant anti-inflammatory action in comparison to control and standard analgesic drug. Amongst various synthetic compounds, new chromane and SI exhibited maximum anti-inflammatory activity in terms of increment of paw volume and % inhibition of edema (paw) at 100 mg/kg during 3 h of the study. On the other hand, animals treated with SV-SX showed mild anti-inflammatory action in terms of increment of paw volume and %age inhibition of edema in paw at the 100 mg/kg dose, p.o. during 3 h of the study in comparison to control as well as standard drug.

Table 5: Anti-inflammatory activity of test compounds (new chromane and SI-SX) vs diclofenac sodium (Standard)

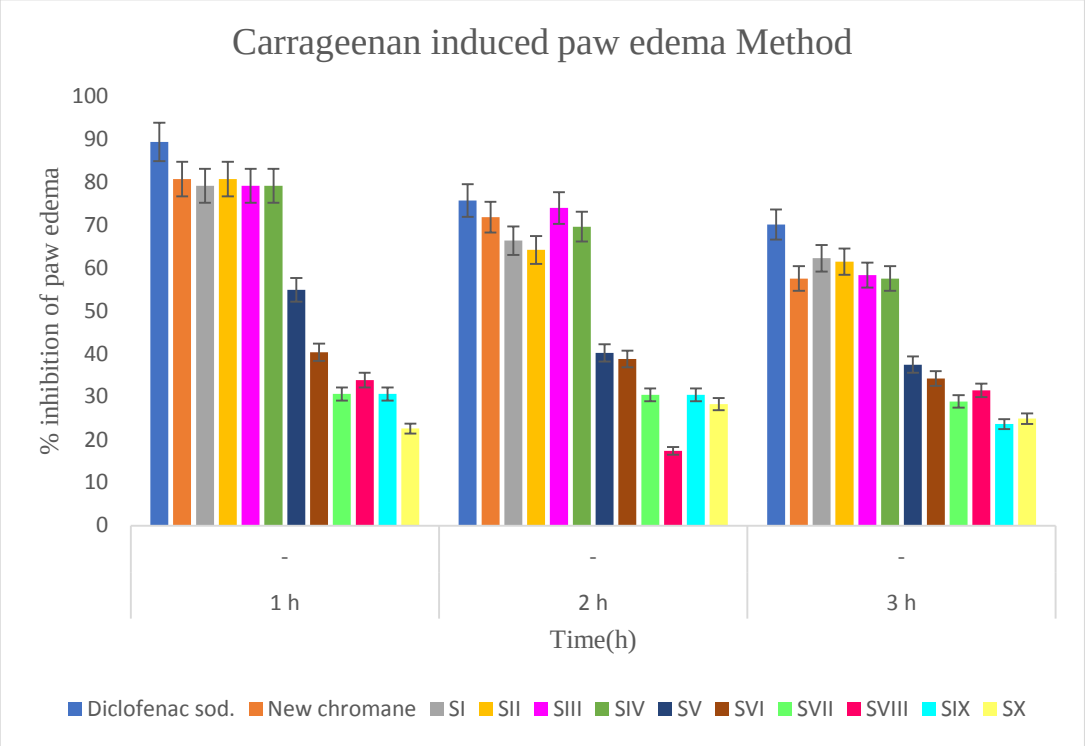
% inhibition = T_o (Control) − T_t (Test) / T_0 (Control) ×100

Treatment	Dose (mg/kg)	Increment in paw volume (ml) Meanvalue ± Standard deviation			% age of paw edema		
		1 h	2 h	3 h	1 h	2 h	3 h
Control	Vehicle	0.62 ± 0.01[a]	0.92 ±0.01[a]	1.27 ±0.05[a]	-	-	-
Diclofenac	100	0.11 ±0.00*	0.27 ±0.03*	0.52 ±0.02*	89.25	75.65	70.06

sod.							
New chromane	100	0.12 ±0.01*	0.26 ±0.04*	0.54 ±0.05*	80.64	71.74	57.48
SI	100	0.13 ±0.01*	0.31 ±0.02*	0.48 ±0.05*b	79.03	66.30	62.20
SII	100	0.12 ±0.01*	0.33 ±0.02*	0.49 ±0.09*ab	80.64	64.13	61.42
SIII	100	0.13 ±0.01*	0.24 ±0.02*	0.53 ±0.02*	79.03	73.91	58.27
SIV	100	0.13 ±0.01*	0.28 ±0.04*	0.54 ±0.04*b	79.03	69.56	57.48
SV	100	0.28 ±0.02*ab	0.55±0.04*ab	0.92 ±0.04*ab	54.84	40.22	37.46
SVI	100	0.37 ±0.02*ab	0.60±0.04*ab	0.95 ±0.05*ab	40.32	38.78	34.20
SVII	100	0.43 ±0.01*ab	0.64±0.06*ab	1.03 ±0.06*ab	30.64	30.43	28.90
SVIII	100	0.41 ±0.01*ab	0.76±0.02*ab	0.87 ±0.06*ab	33.87	17.39	31.49
SIX	100	0.43 ±0.02*ab	0.64±0.03*ab	0.97 ±0.06*ab	30.64	30.43	23.62
SX	100	0.48 ±0.02*ab	0.66±0.05*ab	1.03 ±0.04*ab	22.58	28.26	24.90

n = 5; Mean ± S.D.; *P<0.05 vs. control; aP<0.05 vs. standard; bP<0.05 vs. parent compound

Figure3: Anti-inflammatory activity of new chromane, SI-SX (test compounds) vs Pentazocine (Standard)



CONCLUSION

This study was performed for*in-vivo* analgesic and anti-inflammatory investigation of synthesized new chromane and its analogues (SI-SX) following tail immersion, hotplate and carrageenan induced paw edema method respectively. Before *in-vivo* biological evaluation oral acute testing was done on the testing animals revealed, no mortality (LD_{50}) and other effects were observed for next 7-14 days.

In analgesic study (*in-vivo*), Tail immersion method revealed that new chromane and SI showed significant analgesic effect w.r.t reference whereas SV-SX showed mild analgesic effect. Hot plate method revealed that compound new chromane and SI showed maximum analgesic activity in terms of significantly increased tail flicking response timefollowed by

new chromane, SIII, SII, SIV and SV at the same dose during 2 h of the study and SVI-SX showed less analgesic effect in comparison to reference drug.
Amongst various synthetic compounds, new chromane and SI exhibited maximum anti-inflammatory activity in terms of increment of paw volume and % inhibition of paw edema. On the other hand, animals treated with SV-SX showed mild anti-inflammatory action in terms of increment of paw volume and percentage inhibition of paw edemain comparison to control as well as standard drug.
Therefore,*in-vivo* evaluation revealed, new chromane and O-alkyl substituents (specifically SI) were found more potent than others because O-CH_3 substitution enhance the metabolic stability of chroman-4-one skeletonmoreover, improve physicochemical properties of compounds. [17-18]

CONFLICT OF INTEREST

Authors declare no conflict of interest.

REFERENCES

1. Howard R, Carter B, Curry J, Morton N, Rivett K, Rose M, *et al.* Good practice in postoperative and procedural pain management. PaediatrAnaesth. 2008;18(s1):1-3.
2. Kumar M, Shete A, Akbar Z. A Review on Analgesic: From Natural Sources. Int JPharm BiolSci Arch.2010;1(2):95-100.
3. Walker JS. NSAID: An update on their analgesic effects. Clin Exp Pharmacol Physiol.1995;22(11):855-60.
4. Donnelly MT, Hawkey CJ. COX-II inhibitors-a new generation of safer NSAIDs? Aliment PharmacolTher.1997;11(2):227-36.
5. Zarghi A, Arfaei S. Selective COX-2 Inhibitors: A review of their structure activity relationships. Iran J PharmRes. 2011;10(4):655-83.
6. Kitahiro Y, Koike A, Sonoki A, Muto M, Ozaki K, Shibano M. Anti-inflammatory activities of *Ophiopogonisradix* on hydrogen peroxide-induced cellular senescence of normal human dermal fibroblasts. J Nat Med. 2018;72(4):905-14.
7. Kamboj S, Singh R. Chromanone-A Prerogative Therapeutic Scaffold: An Overview. Arab JSci Eng. 2022; 47:75–111.
8. Begum AF, Balasubramanian KK, Shanmugasundaram B. 3-Arylidene-4-Chromanones and 3-Arylidene-4-Thiochromanones: Versatile Synthons towards the Synthesis of Complex Heterocycles. Asian J Org Chem. 2022: e202200328.
9. Gazak R, Sedmera P, Vrbacky M, Vostalova J, Drahota Z, Marhol P *et al.* Molecular mechanisms of silybin and 2, 3-dehydrosilybin antiradical activity-Role of individual hydroxyl groups. Free Radical Bio Medi. 2009;46(6):745-58.
10. Kaur N, Kishore L, Singh R. Antidiabetic effect of new chromane isolated from *Dillenia indica* L. leaves in streptozotocin induced diabetic rats. J Funct Foods. 2016; 22:547-55.
11. Kamboj S, Sharma M, Singh R. Synthesis and molecular docking studies of new chromane (2-(4-hydroxybenzyl) 3,5,7-trihydroxychroma-4-one) and its O-substituted analogues. Int J Health Sci, (2022);6(S4):11899–11916.
12. Singh AK, Saha S. Chemistry, therapeutic attributes, and biological activities of *Dillenia indica* Linn. Environ. Biotechnol.: for sustainable future. Springer, Singapore.2019:237-260.
13. Yan J, Sun LR, Zhou ZY, Chen YC, Zhang WM, Dai HF, Tan JW. Homoisoflavonoids from the medicinal plant *Portulaca oleracea*. Phytochem. 2012; 80(8):37-41.
14. Kiruthiga N, Alagumuthu M, Selvinthanuja C, Srinivasan K, Sivakumar T. Molecular modelling, synthesis and evaluation of flavone and flavanone scaffolds as anti-inflammatory agents. Antiinflamm Antiallergy Agents Med Chem. 2021;20(1):20-38.

15. Scheffer WC. Statistics for the Biological Sciences. Philippines: Addison-Wesley Publishing Company.1980:121-141.
16. Li NG, Shi ZH, Tang YP, Yang JP, Duan JA. An efficient partial synthesis of 4′-O-methylquercetin via regioselective protection and alkylation of quercetin. Beilstein J OrgChem. 2009;5(1):60.
17. Shi ZH, Li NG, Tang YP, Shi QP, Tang H, Li W, *et al*. Biological evaluation and SAR analysis of O-methylated analogs of quercetin as inhibitors of cancer cell proliferation. Drug Dev Res. 2014;75(7):455-62.
18. Jonsson M, Jestoi M, Nathanail AV, Kokkonen UM, Anttila M, Koivisto P, *et al*. Application of OECD Guideline 423 in assessing the acute oral toxicity of moniliformin. Food ChemToxicol. 2013; 53:27-32.