Formulation and screening of analgesic activity of different analgesic gel preparations

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Abstract: This study was aimed to investigate the dermal irritant test and the central analgesic effects of topical application of seven different formulations (A-G) of analgesic gel prepared from locally available raw material in animal model. The results of dermal irritant test revealed that no animal showed any kind of toxic effect i.e. redness, irritation, itching, inflammation, skin infection or any other injurious effects. All animals remained healthy, active, alert showing normal behavior and no mortality was observed during the claimed period. The analgesic activity was performed by tail flick test. The analgesic activity against tail flick test revealed that all samples of test gel had analgesic effect at 15, 30 and 60 minutes after sample application but sample D had highest analgesic effects (193%) followed by sample E (155%), sample C (122%), sample G (85%), sample B (84%),sample F (81%) while sample A exhibited (73%) analgesic activity. Wintogeno was run as standard drug and it showed 168% analgesic activity.

Keywords: salicylate derivatives, rats and rabbits dermal irritant test, analgesic test.

INTRODUCTION

Pain can be defined as an unpleasant sensory and emotional experience associated with tissue injury. Tissue damage is directly associated with instant pain as it releases certain chemical mediators (prostaglandins, bradykinins) that causing pain sensation (Banerjee *et al*., 2012). A drug that bringing about insensibility to pain without loss of consciousness is called analgesic or pain killer (Ezeja *et al*., 2011). Analgesics are use either orally or topically in the form of tablets, gels, ointments etc. Usually to get relief from acute and chronic pain, oral pain killers are prescribed but sometime systemic adverse effects may observed associated with these pain killers (Shylaja *et al*., 2008). As compare to oral analgesics topical analgesics were more potential with minimal adverse systemic effects and provides same analgesic relief (Argoff, 2013). The advantage of topical or local application of analgesics or anesthetics over the targeted or painful site includes ideally to produce an effective action in reducing pain with minimal systemic absorption by target the peripheral nerves and soft tissue, less side effects, painless drug delivery, more adherence and acceptance for patients, ease of dose termination and avoidance of first pass metabolism (Tadicherla, 2006). In topical analgesic preparations use of menthol and thymol is very common because of their reported analgesic effects (Beer *et al*., 2007; Haeseler *et al*., 2002). Worldwide researchers are busy to formulate topically acting agents and improve their absorption across the main barrier of the skin. In the light of all these facts we decided to work on some new laboratory prepared topical analgesic agents in scientific manner that can be use in long term with no significant systemic accumulation, minimizing risks of adverse effects and to boost the existing information.

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MATERIALS AND METHODS

Ingredients used in formulation

Menthol (3-p-Methanol), Thymol (Thyme camphor), Carboxy polymethylene were used of commercial grade.

Preparation of salicylate derivatives

4-Acetoxybenzoic Acid: 5g of 4-hydroxybenzoic acid was taken in a 100ml two necked round bottom flask. 10ml ethyl acetate followed by 5ml acetic anhydride and few drops of concentrated sulfuric acid were added to the flask. It was then heated in a water bath on a hot plate with magnetic stirrer with constant stirring for one hour at 50-60^{o}C. Add sufficient amount of water, while precipitates formed. It was then filtered and dried in a vacuum oven at 60^{o}C. It is used in formulation B and C of Anagel. 2 acetoxy benzoic acid (Aspirin) Acetyl Salicylic Acid 10g salicylic acid was taken in a 100ml two necked round bottom flask. 15ml acetic anhydride and few drops of concentrated sulfuric acid were added into it. The flask was heated in a water bath on hot plate with magnetic stirrer with constant stirring for 30 minutes at 50-60^{o}C. Add sufficient amount of water, while precipitates formed. Filtered and dried in a vacuum oven at 60^{o}C. It is used in formulation D and E of Anagel.

Methyl salicylate (oil of wintergreen)

4g of salicylic acid was reacted with 6ml methanol on heating with constant stirring at 60-65^{o}C for 1½ hour to give oil of wintergreen which is transparent liquid and have good fragrance of balm. It is used in formulation F and G of Anagel.

Preparation of anagel (A)

0.6g Carboxypolymethylene was treated with aqueous solution of sodium hydroxide (0.165g) and *q.s.* to 100ml water with constant stirring. Then a mixture of thymol (1.8g) and menthol (1.7g) in isopropanol (1.8g) were added subsequently to the above solution and stirred at room temperature for five hours to obtain a gel preparation, then volume make up with water.

Preparation of anagel (B-G)

0.6g Carboxypolymethylene was treated with aqueous solution of sodium hydroxide (0.165g) and *q.s.* to 100ml water with constant stirring. Then a mixture of thymol (1.8g) and menthol (1.7g) in isopropanol (1.8g) and 4-acetoxy benzoic acid (0.5%) were added subsequently to the above solution and stirred at room temperature for five hours to obtain a gel preparation Anagel B. Similarly formulations C-G have been prepared by substituting the respective salicylic acid derivative (table 1).

Analgesic activity

Animals

Either sex of albino rabbits (1.5kg) and wistar strain rats (150-200g) were obtained from Animal house of PCSIR Labs Complex Karachi. These animals were housed under standard laboratory conditions and fed with standard pellet diet with water *ad libitum*. All the experimental procedures and protocols used in the study were reviewed by the Institutional Animal Ethics Committee of PCSIR. The animals were deprived of food for 24 hour before experimentation but allowed free access to water throughout.

Dermal irritant test

The degree of dermal irritation of all laboratory formulation were measured on the intact skin of the albino rabbits (n=10). Seven groups of rabbits were made and their fur was clipped in the right and left area on the dorsal side of each rabbit one day before the test. The left side served as a negative control, while the right one served as a test site. A thin layer of sample A, B, C, D, E, F and G was applied on the right side of each animal of their respective group. The test area was covered with a gauze patch, plastic sheet and a non-irritant adhesive plaster. The control sites were treated with gel base and covered as above. After 24h of exposure, the coverings were removed and the test site rinsed with distilled water and dried. The animals were examined for the presence of erythema and edema according to the Draize dermal irritation scoring system (0, no erythema or no edema; 1, barely perceptible erythema or edema; 2, well defined erythema or slight edema; 3, moderate to severe erythema or moderate edema; 4, severe erythema or edema) at grading intervals of 24, 48 and 72h after topical application to find out the dermal toxicity (Nair *et al.*, 2014, Njateng *et al.*, 2013)

Analgesic testing

The animals were tested for tail flick by Analgesiometer (UGO Basile, Itlay) as it was described earlier (Rahman *et al.*, 2015). Animals were divided into nine groups (n=5). Group I-VII treated as test groups and received topical application of sample A, B, C, D, E, F and G respectively. Group VIII served as standard group and received Wintogeno application as standard drug while Group IX served as control group and received gel base application only. In tail flick test each animal was held in suitable restrainer with the whole tail extending out. The samples were applied topically to the tail of respective groups 15 minutes before taking response on the upper half of the tail. The intensity of heat was adjusted at 5 Ampere. The cut off time was set at 15 seconds to prevent any tissue damage. The time (in second) required for the animal to withdraw (flick) its tail from the heat source was measured. The results were noted initially at 0.0 minutes (Tb) and at the intervals of 15, 30 and 60 minutes (Ta) after the animals were treated locally. Percentage analgesic activity was calculated as per formula shown below:

$$\%\ \text{of analgesic activity} = \frac{\text{Ta - Tb}}{\text{Tb}} \times 100$$

STATISTICAL ANALYSIS

Values for analgesic activity were expressed as mean ±S.D. Statistical significance of the difference was assessed by student's t-test values of $p<0.05$ were considered as significant and $p<0.0001$ as highly significant.

RESULT

Dermal irritant test

The dermal irritation test of thin layer of test and control samples not exhibited any kind of toxic effect i.e. redness, irritation, itching, inflammation, skin infection or any other injurious effects. All the animals remained healthy, active, alert showing normal behavior during the claimed observation period and no mortality was observed. This shows that all samples were free from toxic effects (table II).

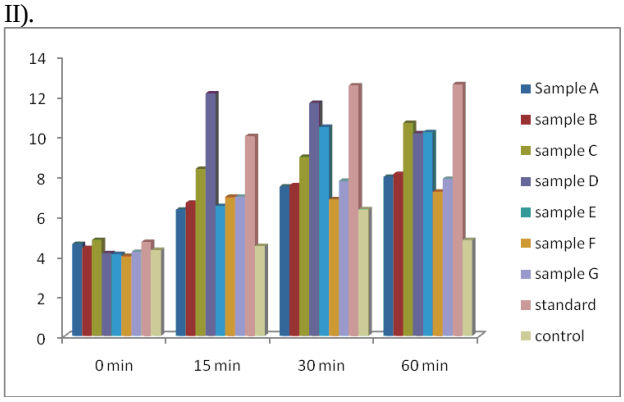

Fig. 1: Analgesic activity test by tail flick method

Table 1: Compositions of formulations

Sr. no.	Sample	Name of the Ingredient
1.	A	Anagel
2.	B	Anagel + 4-Acetoxybenzoic acid 0.5%
3.	C	Anagel + 4-acetoxybenzoic acid 1%
4.	D	Anagel + Acetyl salicylic acid (2 acetoxy benzoic acid) 0.5%
5.	E	Anagel + Acetyl salicylic acid (2 acetoxy benzoic acid) 1%
6.	F	Anagel + Methyl salicylate (Oil of Wintergreen) 0.5%
7.	G	Anagel + Methyl salicylate (Oil of Wintergreen) 1%
8.	Standard	Wintogeno ointment
9.	Control (Blank)	gel base (thickening agent + alkali)

Table 2: Dermal irritant test of test formulations

Sr. #	Group	No. of test animal	Sex ratio	Toxic effect
1.	A	10	5:5	Nil
2.	B	10	5:5	Nil
3.	C	10	5:5	Nil
4.	D	10	5:5	Nil
5.	E	10	5:5	Nil
6.	F	10	5:5	Nil
7.	G	10	5:5	Nil

Table 3: Analgesic Activity of test formulations

Sr. #	Sample	Pre drug reaction Time (0 min)	Tail flick time (sec)		
			15 min	30 min	60 min
1.	A	4.6±0.49	6.32±0.43** (37%)	7.48 ±0.37* (63%)	7.96±0.60* (73%)
2.	B	4.4±0.46	6.66±0.51** (51%)	7.54±0.71* (71%)	8.1±0.54* (84%)
3.	C	4.8±0.39	8.36±0.27** (74%)	8.96±0.38* (87%)	10.66±0.23** (122%)
4.	D	4.14±0.45	12.14±0.39** (193%)	11.66±0.54** (182%)	10.14±0.62** (145%)
5.	E	4.1±0.38	6.5±0.21** (59%)	10.46±0.32** (155%)	10.2±0.48** (149%)
6.	F	4±0.07	6.96±0.20 ** 74%)	6.84±0.21 (71%)	7.22±0.31* (81%)
7.	G	4.2±0.61	6.96±0.46** (64%)	7.76±0.20* (83%)	7.86±0.32* (85%)
8.	Standard	4.7±0.30	10±0.4**(113%)	12.54±0.34** (167%)	12.6±0.30** (168%)
9.	Blank	4.3±0.68	4.5±0.32	6.34±0.83	4.8±1.54

Analgesic test

The antinociceptive or analgesic activity of topical application of seven different test formulations, standard drug and blank sample was assessed using Tail flick method. The results obtained after 15 minutes shows that all test samples had strong analgesic activity (table III) but sample D (193%), E (155%) and C (122%) had more potent and highly significant pain inhibiting activity while samples G (85%), B (84&) F (81%) and A exhibited (73%) analgesic activity. Standard drug Wintogeno showed gradually increase analgesic activity with the period of time (168%).

DISCUSSION

According to WHO health can be defined as a complete state of physical, mental and social well-being and not merely the absence of disease or infirmity" (Matthew *et al.*, 2013). Pain is unwanted feeling with which the affected person wants to get rid off as soon as possible. Analgesics are agents use for pain relieving. A huge number of analgesic preparations (oral and topical) are available in market for treatment and management of musculoskeletal injuries and disorders. Number of peoples commonly used non prescribed topical analgesics in the form of gels, ointments, creams, lotions, sprays, patches or as single entity or combination formulations. These products may have local analgesic or anesthetic effects (Wright 2006). Anagel plain and Anagel with salicylate derivatives were prepared in seven different combinations on laboratory scale using locally available raw material (table 1). These samples of gels were tested for dermal irritant effects and for analgesic activity. The dermal irritation test revealed a negligible irritant effect on rabbits skin (table II). No animal showed any kind of toxic effect or signs of inflammation i.e. redness,

irritation, itching, any skin infection or any other injurious effects. All animals remained healthy, active, alert showing normal behavior and no mortality was observed during claimed observation period. Tail flick method was used to evaluate the analgesic activity of topical application of test and standard drugs at 15, 30 and 60 minutes respectively. The results of analgesic activity revealed that the all gels had strong analgesic effect. The sample Anagel+Acetyl salicylic acid (2 acetoxy benzoic acid) 0.5% (sample D) showed maximum potent analgesic effects at 15 minutes (193%) after drug application followed by sample E (155%), sample C (122%), sample G (85%), sample B (84%) and sample F 81%, while sample A exhibited 73% analgesic activity. The commercially available drug Wintogeno showed gradually increased analgesic activity (168%) but the duration of analgesia was more than test samples (table III, fig. 1). It is well reported that topical application is more suitable than orally as it penetrates skin, absorbed into tissue and inhibit enzyme cyclo-oxygenase and causes pain relief. The topical preparations effects only affected area and not systemically absorbed (Rahman *et al*., 2015). The Radiant heat tail flick method has been found to be suitable for the evaluation of centrally acting analgesics. It involves higher brain functions and consists of responses to nociceptive stimuli organized at a supra spinal level (Desai *et al*., 2010). A search from internet shows that menthol and thymol (the main ingredients of our preparation) are used in many analgesic preparations (Pan *et al*., 2012, Sundstrup *et al*., 2014). It is reported that menthol reduces acute pain, neuropathic pain, and inflammatory pain (Swandulla *et al*., 1987; Gaudioso *et al*., 2012). To relieve pain and swelling acetylsalicylic acid (ASA) and other non-steroidal anti-inflammatory drugs (NSAID) were also used but salicylic acid is most important analgesic (extensively used for headache, inflammation, arthritis pain etc). Thymol and salicylic acid irreversibly inhibits the enzyme cyclo-oxygenase (COX-1 and COX-2), an enzyme that is responsible for the production of pro-inflammatory mediators such as the prostaglandins and its derivatives that cause inflammation (Adams and Wang 2015; Azuma *et al*., 1986; Ahmed *et al*., 2011). All these studies support our results. It was observed that all gel preparations containing salicylic acid derivatives show better analgesic activity than the base compound "Anagel". These experiments demonstrate that incorporation of analgesics in gel preparation effectively increase its potency. Salicylic acid derivatives containing free COOH group and alkylated at OH group show better analgesic activity than methyl salicylate which has free OH group, when similar concentration of active ingredient is used. 2-Acetoxy benzoic acid having OH group at 2^{nd} position show better activity than 4-acetoxy benzoic having free OH at 4^{th} position. It shows that there is a stereo-chemical aspect of the analgesic activity. It is well reported that 2-hydroxybenzoic acid is used to ease ache (Madan and Levitt 2014; Fadeyi *et al*., 2004), therefore our results are justified. Results also showed that the greater amount of 2-Acetoxy benzoic acid i.e. 1% has less activity means it is effective when it is applied in amount equal to maintains requirement that is 0.5% 2-Acetoxy benzoic acid. It is reported that topical application of preparations in small amount can be used over skin where they are required and the analgesic molecules in these topical preparations penetrate the skin in small but sufficient amounts, act where they are required and are quickly cleared from the skin and the body (Adams and Wang 2015). ASA is originally derived from the plant sources, and today according to WHO survey 80% world's population relay on natural medicines (Shylaja *et al*., 2008; Zulfiker *et al*., 2010). According to fadeyi *et al*., (2004) Aspirin produce analgesia by both peripheral and central action similar to other NSAIDs. In the light of these facts our formulation can highly be acceptable. Therefore now in modern medicine, salicylic acid and its derivatives are used as constituents to soothe joint and muscle pain.

CONCLUSION

The above studies show that different samples of the test drug possess significant topical analgesic activity and further work can be carried out to develop a cost effective drug, as the drugs available in market for this purpose are too expensive for common man, which could be a good source of external pain reliever with least side effects.

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