EVALUATION OF ANTI-INFLAMMATORY EFFECT OF IBUPROFEN USING WITEPOSOL H15 RECTAL SUPPOSITORIES VERSUS ORAL ROUTE

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ABSTRACT

Inflammation is the self-protective reaction of tissues towards infection, irritants, or foreign substances. Though it is a part of host defense mechanism, when it becomes severe, it turns out to be a hopeless condition which causes damage of tissues; hence control of inflammation becomes essential. This paper presents a study of anti-inflammatory effect of ibuprofen-witepsol H15 rectal suppositories versus oral route of administration using intra-peritoneal (IP) route as positive control. Suppositories were prepared using fusion method and tested for physico-chemical properties according to British Pharmacopeia (B.P) 2007 procedures. Screening for anti-inflammatory activity of ibuprofen was carried out against carrageenan-induced rat paw oedema in wistar strain albino rats (200 ± 20 g) of either sex. The formulated suppositories were found to satisfy B.P, requirements for weight variation, liquification time, disintegration time, hardness values and content uniformity. The entrapment efficiency (%) of ibuprofen was 99-100 % w/w for all the tested suppositories. The increase in paw oedema volume significantly ($p< 0.0001$) inhibited using ibuprofen-witepsol H15 rectal suppositories compared to control. The total area under the curve (AUC) for paw volumes time curve were reduced from 5.54 ml/h for control to 3.45, 4.38 and 4.78 ml/h using rectal suppositories, oral and IP routes. The anti-inflammatory inhibition (%) achieved after 4-5 hours was 40.58, 29.87 and 65.87, after rectal suppositories, oral and I.P routes of administration respectively. The total AUC (%) of inhibition were 33.81, 27.07 and 62.16 at 4 and 5 hours. using, rectal suppositories, oral and IP routes of administration respectively. The results justified the use of suppositories in the treatment of inflammation when oral route is unreliable.

KEY WORDS: Ibuprofen, Anti-inflammatory activity, Suppository base, Oral route, Paw oedema

INTRODUCTION

Inflammation is the self-protective reaction of tissues towards infection, irritants, or foreign substances[(1)]. It is a part of host defense mechanism, when it becomes severe, it turns out to be a hopeless condition which causes damage of tissues. Chronic inflammation is associated with certain severe disease like rheumatoid arthritis, type II diabetes, Alzheimer's disease[(2)]. Non-steroidal anti-inflammatory drugs (NSAIDs) are the available potent synthetic drugs in the treatment of inflammatory diseases but[(3)]. The prolonged oral uses of NSAIDs are well known to be associated with peptic ulcer formation. Rectal route using suppositories can be a promising alternative approach for administration of these drugs. Rectal route provides reduction of side effects namely gastrointestinal irritation and the avoidance of both disagreeable taste and first pass effect[(4-7)]. Rectal administration can also be an alternative route when oral route is not possible in nausea, vomiting, and unconscious conditions[(8,9)]. Comparison of the oral administration of ibuprofen with rectal suppositories revealed that ibuprofen suppositories can be considered to management of fever and pain when the oral route is not available[(10)]. In addition, NSAIDs are usually good candidates for the development of conventional or controlled release preparations particularly through the rectal route. Carrageenan rat paw oedema model is traditionally used for search and development of new NSAIDs with assessment of effects after oedema induction[(11,12)] neglecting long-term effects[(13,14)]. Therefore, the aim in this study is to assess the anti-inflammatory activity of ibuprofen recta suppositories in comparison to oral route using intra-peritoneal routes as positive control.

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MATERIAL AND METHODS

Ibuprofen was from Prodotti Chemici Industrial, Italia. Witepsol H15, (WH15) was from Dynamit Nobel, Witen, Germany. Carrageenan sodium was from (BDH, England). Water for injection (2 ml) ampoules was bought from local pharmacy. All other chemicals used in this work were from Sigma-Aldrich Co. (St. Louis, MO, USA) and used as received.

Animals:

Wistar strain albino rats weighing 200 ± 20 g of either sex were used in the study. The animals were maintained on standard diet and tap water. The temperature and humidity were kept at optimum and the animals were exposed to natural day-night cycles. The study was conducted in accordance with the nationally accepted guidelines for laboratory animal use and care which was approved by Animal Ethical Committee (NMRC35/2009).

Preparation of Suppositories:

Suppositories plain and containing 15 mg/kg of ibuprofen were prepared using the fusion method.(15) Briefly, Witepsol H15 base in absence and presence of ibuprofen were melted and poured into six cavities metal mould. The prepared suppositories were left for 24 hrs. at 25^{o}C before testing. Displacement value for

ibuprofen was calculated based on the following equation:

$$f = (100 \times (E - G))/(G \times x) + 1.$$

Where f is the displacement value, E is the weight of the suppository without ibuprofen, G is the weight of the suppository with active substances and x is the active substance content in percentage[16].

Characterization of Suppositories:

Weight Uniformity:

Weight uniformity test was carried out according to British Pharmacopoeia B.P 1998 method[17] Briefly, 20 suppositories were individually weight and the average weight then calculated. There must be not more than two suppositories differ from the average weight by more than 5% and no suppository differs from the average weight by more than 10%.

Liquefaction Time:

The ascending melting point method was used to determine the melting point of each suppository[18]. Briefly, capillary tubes of 10 cm in length sealed at one end were filled with the formulation to about 1cm height. The tubes then dipped in gradually heated electro-thermal thermometer from which the temperature for melting of suppositories was predicted.

Disintegration Criteria:

Disintegration test was performed on six suppositories according to British Pharmacopoeia 2007[19] using USP tablet disintegration (Model PTW, Germany) test apparatus. Disintegration time (D.T.) for suppositories was determined in water maintained at 37± 0.5°C.

Hardness Test:

Hardness test was performed using Erweka hardness tester. The temperature inside the testing chamber was maintained at 25˚C by means of circulating water from thermostat connected to the tester. The suppository was placed into the holding device with the tip upwards and the test chamber was then closed with glass plate. At this point, the initial load, which was given by the entire suspended block, was 600 gm. Subsequently every minute a disk of 200 gm. was added until the suppository crush under the load of the weight. The mass required to crush the suppository was calculated by the summing the masses weighing on the suppository when it was collapsed (including the initial mass of the device i.e. 600 gm.)[20].

Content Uniformity:

Ibuprofen content determination was done using phosphate buffer pH 7.4 as solvent medium. Three randomly selected suppositories from each formulation were taken in 1000 ml flask containing 100 ml phosphate buffer pH 7.4. The flask was shaken until the suppositories completely dissolved. Samples of the resulting solutions were appropriately diluted, filtered through doubled layer Whatman filter paper followed by 0.45 µm disc filter and subjected to absorbance measurement on Shimadzu PR240, Kyoto, Japan UV/Vis spectrophotometer at 264 nm using suppository solution prepared without ibuprofen as a blank. Ibuprofen content was calculated using calibration curve equation obtained by plotting the absorbance for serial concentrations of ibuprofen in the phosphate buffer pH 7.4[21].

Anti-Inflammatory Studies:

The method of Winter *et al*.[22] was adopted to screen the anti-inflammatory activity of ibuprofen against carrageenan-induced paw oedema in Wistar strain Albino rats. Prior to start of the experiment, the body weight of animals was recorded individually for evaluating proper treatment dosage and animals, divided into four groups of six each. Group I received distilled water and served as -ve control. Group II received the test drug, ibuprofen by oral route at dose of 2 mg/ml in water. Group III: rats received intraperitoneal (I.P) injection of ibuprofen (2mg/ml) in water for injection and served as +ve control. Animals in Group IV, received treatments with rectal WH15-ibuprofen suppositories respectively. After one hour, 50 µl of freshly prepared 1% λ-carrageenan sodium in saline was injected into the sub-plantar region of the right hind limb of the tested rats. Paw volume was measured by volume displacement method using a digital plethysmometer (Ugo Basile, Italy) immediately and after 0.5, 1.0, 2.0, 3.0, 4.0, 0.5, 6.0 and 8.0 hour of λ-carrageenan sodium injection. A significant reduction in the paw volume compared to untreated control animals was considered as the in vivo anti-inflammatory response.

Calculation:

The change in paw oedema volume for each time interval was calculated using the following equation:

$$\text{Volume of oedema} = V_f - V_i$$

where V_f and V_i are the volumes of the paw oedema after and before Carrageenan sodium injection. The percentage (%) of inhibition of inflammation for each time interval was calculated applying the following equation:

$$\%\ \text{inhibition of inflammation} = \text{NC-T /NC x } 100$$

where NC refers to negative control and T refers to the test groups.

The area under the curve for oedema volume curve of the time-course was calculated using the trapezoidal rule.

$$AUC = \sum_{i=1}^{n} \frac{(Vi\text{-}1 + Vi)}{2} (ti - ti\text{-}1)$$

where V_i is paw oedema volume at t_i

The level of inhibition response of inflammatory was calculated using AUC according to the following relation:

$$\%\ \text{Inhibition. of. oedema} = \left(\frac{AUC_{control} - AUC_{test}}{AUC_{control}}\right) \times 100$$

Statistical Analysis:

Results of anti-inflammatory activity were expressed as Mean increase in paw diameter ± SEM (standard error of the mean). Results were analyzed using one-way analysis of variance (ANOVA) followed by t test using Graph Pad software 2016 Quick Calcs. Differences were considered as statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

In this work, ibuprofen-suppositories were formulated using Witepsol H15 as fatty base. The formulated rectal suppositories were studied for anti-inflammatory activity of ibuprofen in comparison to oral administration using IP route as positive (+ve) control. (Table 1) shows the preliminary physico-chemical evaluation results of suppositories using British Pharmacopoeia (BP) 2007 procedures.

(Table 1) Physicochemical Characterisation data of Ibuprofen Witepsol H15 Suppositories.

Formula	Weight mg± SD n=20	Liquefaction Time (min)	Hardness (Kg)	Disintegration Time (min)	Content Uniformity (mg) ± SD	Entrapment efficiency (%w/w)
WH15	1.70 ± 0.20	9.0	2.4	11	0.00	0.00
WH15+Ibuprofen	1.69 ± 0.05	8.5	2.0	9	300.2 ± 0.76	100.00

WH15=Witepsol H15,

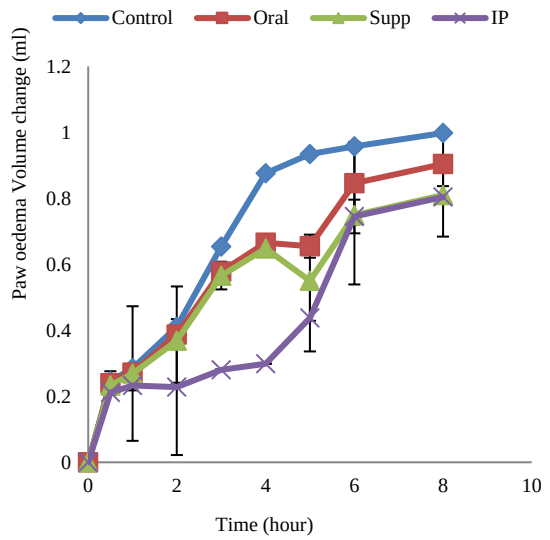
The weight of suppositories for different formulations was found to comply with British Pharmacopoeia (BP) standards[19]. The percentage deviation in weight of all prepared suppositories was less than 0.5 from the average weight. Liquefaction time was ≤ 9.0 min for the formulated suppositories. This value is within the acceptable limit (30 minutes) required for complete melting[17]. Hardness values for the tested suppositories were ≤ 3 kg/cm^2 indicating good mechanical strength with an ability to withstand physical and mechanical stress condition while handling. Disintegration time was 11 min for plain suppositories reduced to 9.0 min after incorporation of ibuprofen. The content of ibuprofen in different suppository formulations was highly uniform with the entrapment efficiency (%) was 99-100 % w/w that is within the limits (95-105%) specified by British Pharmacopeia, BP[17]. Carrageenan induced paw oedema is a well-established model to test effect of drugs against acute inflammatory response[23]. The inflammatory response generated in this model is considered to be biphasic in nature with a preliminary oedema generation caused primarily by the effect of hypersensitivity mediators like histamine, serotonin and bradykinin on vascular endothelium, which is followed by a delayed response by the release of prostaglandins and nitric oxide[24]. The model have been used by several investigators to search for new anti-inflammatory product[25,26]. In the present work the model was applied to evaluate the anti-inflammatory activity of ibuprofen-rectal suppositories in comparison to oral ibuprofen using IP route as a positive control. (Table 2) illustrates the change in the paw oedema volume for each group of animals and the percentage inhibition with the treatment compared to control.

(Table 2) Paw oedema volume changes and Anti-inflammatory efficiency of deferent ibuprofen formulations.

Treatment	Change in paw oedema volume (ml) and inhibition (%) at respective time (hour) ± SEM							
	0.5	1	2	3	4	5	6	8
-ve control	0.245 ± 0.076	0.285 ± 0.017	0.410 ± 0.005	0.654 ± 0.0046	.876 ± 0.006	0.934 ± 0.010	0.957 ± 0.027	0.998 ± 0.038
Oral Route	0.240 ± 0.036 (2.04)	0.272 ± 0.026 (4. 56)	0.387 ± 0.146 (5.61)	0.578 ± 0.023*** (11.62)	0.665 ± 0.009*** (24.1)	0.655 ± 0.035*** (29.87)	0.846 ± 0.125 (11.60)	0.904 ± 0.102 (9.42)
I.P (+ve control)	0.211 ± 0.006*** (13.9)	0.233 ± 0.015** (18.24)	0.228 ± 0.206 (44.4)	0.280 ± 0.002*** (57.18)	0.299 ± 0.001*** (65.87)	0.437 ± 0.101*** (53.21)	0.745 ± 0.051*** (22.15)	0.804 ± 0.12** (19.44)
WH15 supp.	0.233 ± 0.021 (4.9)	0.269 ± 0.204 (5.6)	0.369 ± 0.001*** (10)	0.566 ± 0.042** (13.46)	0.648 ± 0.004*** (26.02)	0.550 ± 0.121*** (40.58)	0.749 ± 0.21* (21.73)	0.810 ± 0.027*** (18.84)

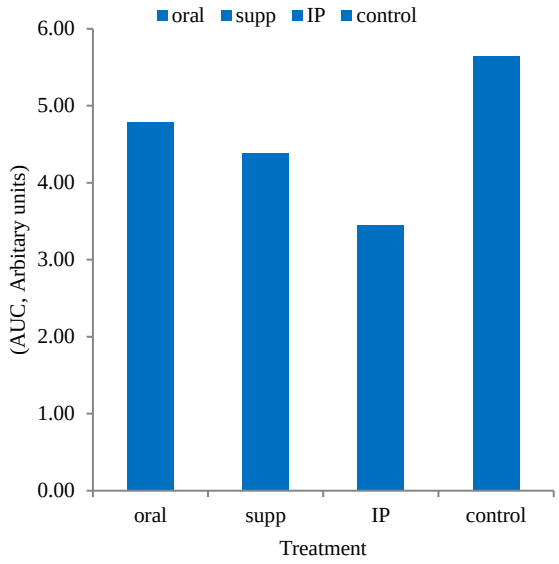
Values are expressed as the mean and ± SEM (n=6) while values in parenthesis represent the percentage inhibition of paw oedema *p<0.05, **p<0.01, ***p < 0.0001compared with vehicle control (T test) WH15, Witepsol H15. -ve control (without Ibuprofen). +ve control (Intra-peritoneal, IP) rout.

All the treated ibuprofen formulations showed significant (<0.05) anti-inflammatory activity reflected in the significant reduction of the oedema volume compared to control. The paw volume was significantly reduced (p<0.0001) after 0.5, 2 and 3 hours following IP injection, rectal suppositories and oral administration of ibuprofen. The change in paw oedema volume measured at 8 hours of administration of ibuprofen was 0.998 ± 0.038 ml (control) reduced to 0.810 ± 0.027 ml (rectal suppositories), 0.904 ± 0.102 ml (oral) and 0.804 ± 0.12 ml (IP). The results showed that before 2 hours post-ibuprofen suppository treatment, unlike IP route which produced significant reduction in oedema; there was no significant difference between the paw oedema in the ibuprofen-rectal suppository, oral treated rats and control. However, from 2 up to 8 hours post-treatment, there were reductions in paw oedema, reaching statistical significance ($p < 0.05$) using rectal suppository compared to control. In contrast, ibuprofen given by oral route showed significant reduction in oedema at 3, 4 and 5 h post treatment but become insignificant at 6 and 8 hours of administration. IP ibuprofen pretreatment, however, produced more significant (Student's *t*-test) reduction in oedema formation in rats compared to the rectal suppository. Ibuprofen suppositories showed stronger inhibition of oedema, as compared to oral route and closely to IP route at the end of six hours (figure 1).



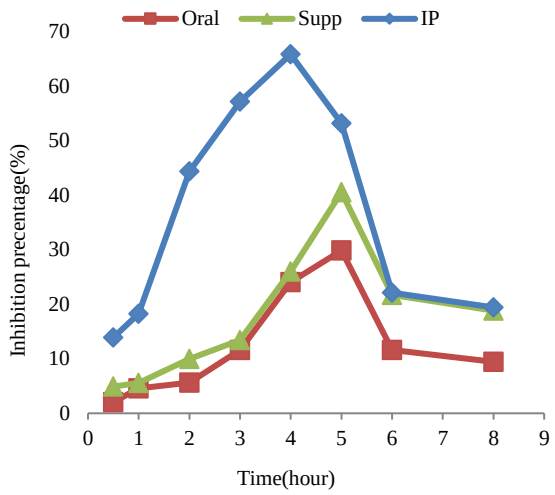
(Figure 1) Paw oedema volume changes after oral, rectal suppository (Supp.) and Intra-peritoneal (IP) administration of ibuprofen. Values are expressed as mean ± SEM, n = 6, *p < 0.05 compared to control with all the groups.

Based on the total AUC for the paw volume changes time curve for the tested formulations, the routes of administration can be ranked in the descending order of IP> rectal > Oral (figure 2).



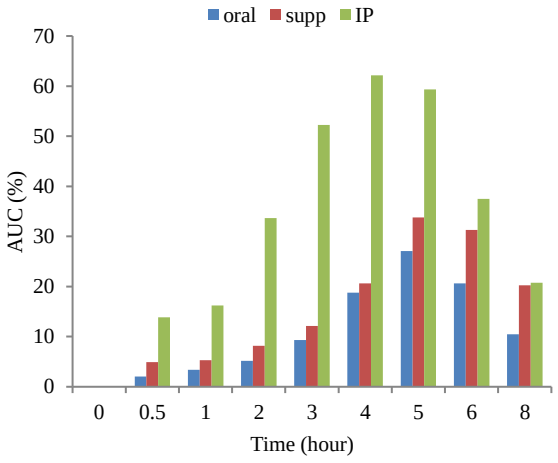
(Figure 2) The calculated AUC for volume change time curve up to 8 h after induction with carrageenan following oral, rectal suppository (Supp.) and Intra-peritoneal (IP) administration of ibuprofen.

The AUC was reduced from 5.54 ml/hour for control to 3.45, 4.38 and 4.78 ml/hour after IP, rectal and oral route of administration. The maximum percentage of inhibition calculated based on the paw volume changes data was 65.87 (%) at 4 hours of treatment using IP, 40.58 (%), at 5 hours using rectal suppositories and at 29.87 (%) using oral route (figure 3).



(Figure 3) The anti-inflammatory inhibition (%) of ibuprofen after oral, rectal suppositories (Supp) and intra-peritoneal (IP) administration.

The high inhibition (%) for IP administration of ibuprofen can be explained by the availability of the anti-inflammatory drug after IP administration route compared to rectal and oral routes. The delayed absorption of ibuprofen lead to that maximum oedema volume reduction almost occurs after four hours of Carrageenan injection. Ibuprofen suppositories exhibited more prominent inhibition of oedema in the later stages of inflammation i.e. after four hours of treatment compared to oral ibuprofen. Despite the fact that the use of suppository statistically showed no significant differences ($p>0.05$) from oral route for administration of ibuprofen in reducing the paw edema volume up to 4 hours of administration, rectal suppositories showed significantly (<0.0001) inhibition effect even similar to IP route after 4 hours. of administration. Similarly, based on the total AUC measured for the three routes for administration of ibuprofen, the percentage inhibition (%) can be ranked order of IP> rectal > Oral (figure 4).



(Figure 4) Area under curve (AUC) inhibition (%) of ibuprofen after oral, rectal suppository (Supp.) and intra-peritoneal (IP) administration.

CONCLUSION

Inflammation is the self-protective reaction of tissues towards infection, irritants, or foreign substances. Oral administration of anti-inflammatory NSAIDs such as ibuprofen usually associated with side effects including irritation of gastrointestinal tract. Though search for alternative route of administration is justified. Rectal administration of ibuprofen-WH15 rectal suppositories to wistar strain albino rats showed significant inhibition of oedema compared to control and better than oral route. The results justified the use of suppositories in the treatment of inflammation when oral route is unreliable. Carrageenan induced paw oedema method proved to be suitable for predicting the anti-inflammatory of ibuprofen using different routes of administration.

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