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Comparative antinociceptive effect of aspirin and aspirin nanoparticles in semisolid formulae in mice

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Abstract

Aspirin are commonly used analgesic, anti-inflammatory, and anti-pyretic drug in medicine, oral route is the most common one for drug administration as a result it will produce different adverse effects like peptic ulcer, nephropathy, and thrombocytopenia even with low and continuous therapeutic dose, so the alternative topical route is preferable with minimal adverse effects and effective concentration. Therefore, in the present study was to investigate whether the antinociceptive property of aspirin would enhance if used aspirin as nanoparticles after preparing it in several forms (gel, cream and ointment). Thirty-two healthy male mice weighing 30-35 gm. were used in the present study. The animals were divided as a randomized design. Each mouse was treated topically. All drug concentration of aspirin was prepared using gel, cream and ointment as vehicle and topically application on fore and hind paw of experimental animals. Pain was induced by application of hot plate for assessment of latency of pain stimulus. Time from placement to jumping or hind paw licking was recorded as latency of response. The result showed that the median effective concentration (EC50) for analgesic effect of aspirin (gel, cream, and ointment) were 0.848, 0.958 and 1.00% respectively while these EC50s were decrease when used nanoparticles aspirin (gel, cream and ointment) to 0.72, 0.657, and 0.701% respectively. In conclusion, topical applied of aspirin will produce effective therapeutic antinociceptive effects in mice although gel preparation produce a better response followed by cream, then ointment due to pharmacokinetic properties. Also nanoparticle preparation will produce superior response in all forms, whether Nano aspirin is prepared in gel form, cream or ointment.

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Introduction

Acetylsalicylic acid (aspirin) is one of the most common drug used in medicine and it have been used since Hippocrates time for their analgesic, antipyretic, and anti-inflammatory effects (1). The analgesic property was produced by mechanism similar to other non-steroidal anti-inflammatory drugs by inhibition of prostaglandin synthesis through different steps which results finally to the inhibition of cyclooxygenase enzymes (COX) (2,3). These enzymes enhance the production of prostaglandin E2 from arachidonic acid usually COX present in two form, COX-1 which is the constitutive form and COX-2 which is the inducible form, aspirin inhibit both enzymes irreversibly, the COX-2 produce therapeutic effects while COX-1 inhibition leading to irritation of gastric mucosa and affect kidney functions (4). When aspirin administrated orally they are rapidly and completely absorbing in a large degree in small intestine and to lesser degree in stomach, and are accompanied by different side effects like gastric upset, blood loss, and to lesser extent Reys syndrome which is rare but fetal adverse effect (5) As a consequence of various

therapeutic effects of aspirin, it presents in different pharmaceutical form tablet, cream, powder (6). Transdermal delivery system provides an alternative method of administrating that bypasses the gastrointestinal tract and its more acceptable by the patients, non- invasive and safe particularly in long term uses of aspirin. The dermal aspirin provide low bioavailability in addition to the avoidance of COX-1 direct contact which present in gastric mucosa (7). Nanotechnology in pharmacology has been of great interest in current time, for the delivery of different drugs (8,9). The ability of these Nano size range small particle to deliver the drug in faster way has been described previously (10,11). EC_{50} is the concentration required to produce 50% of the wanted response in 50% of population, in addition to LD_{50} is required in monitoring submission of new drug (12,13). EC_{50} seems to be a helpful guide in for physician in selecting starting concentration (14), it is safe to start concentration of the drug under the concentration of EC50 due to the fact that different drug can cause morbidity specially when given to the patient with old age and it is dose related, so close monitoring of the concentration is of great important to assess the adverse effects early (12,15). This study aims to prepare normal aspirin and nano aspirin in several forms (gel, cream and ointment) and determine the median concentration of analgesic effect (EC_{50}) for them and then compare them in better analgesic after using them locally through the skin in mice.

After preparing it in several forms (gel, cream and ointment), the mean concentration of analgesic effect (EC_{50}) was determined for them, and then compared them in terms of analgesia from pain after using them locally through the skin in mice.

Materials and methods

Animals

The study was carried out in the pharmacology lab after approved by scientific committee in depart. of dental basic science, College of Dentistry, University of Mosul. Thirty-two male albino mice weighing 30-35 gm were used in the present study. Mice were housed at 23±2ºC and take enough cycle of light / dark and nourished with good diet (standard) and water ad libitum. Mice were brought to laboratory before 3 hours of experiment to adaptation for new environment. The animal obtained from special animal house in dentistry collage, of Mosul university Iraq.

The drug used was aspirin pure (SDI) The animals were randomly divided to treat topically by different formulation aspirin drug. Mouse was treated with topically. all drug concentration of aspirin was prepared using gel, cream and ointment as vehicle and topically application on fore and hind paw of experimental animals. Pain was induced by application of (Heidolph, MR Hei-standard type, Germany) hot plate. To measurement of latency of pain stimulus. Mice were put on a Hot-plate at constant temperature 55±1ºC. The reaction time determine from putting mouse on hot-plate and to licking or hold of the hind paws or fore. Time of acute off 30 seconds is followed to stop any paws thermal injury (16). Time between putting mouse and hind paw licking was considered as response time.

Formulation of aspirin, aspirin nanoparticles Semisolid preparation

All the raw materials used of aspirin pure powder, aspirin nanoparticles (prepared by attrition method that describe by Rajput (17) base and vehicles were supply from commercial sources of analytical grade for research preparation. The concentration prepared as 0.25, 0.5, 0.75, 1, 1.25, and 1.5% by add 0.25, 0.5, 0.75, 1, 1.25, and 1.5 g of active ingredients in total 100 g of formulation respectively, and stirred to confirm homogeneity and contact uniformity.

Ointment

Was prepared by Fusion methods, via melted the desired amount of the active ingredients together with base and additive (PEG 4000,6000, Tween 80, Propylene glycol and distilled water) in two steps of preparation of oil and liquid phase.

Cream

Prepared as oil in water cream, by dissolve desired amount of the active ingredients in water phase then mixed with oil phase mineral oil, stearic acid and lanolin.

Gels

Were prepared by mechanical cold method. by dissolve desired amount of the active ingredients in water then mixed with glycerin and propylene glycol. Evaluation of The prepared semisolid preparations by various specifications of formulations as pH (pH meter), viscosities(viscometer), spread ability (spreading block) and content uniformity (titration and spectrophotometry) according to manufacture quality control instruction (18).

Evaluation of median analgesic concentration (EC_{50}) of aspirin (gel, cream and ointment)

Fifteen mice were used in our study to evaluate median analgesic concentration (EC_{50}) of aspirin (gel, cream, ointment), by using up and down method (19). The pain threshold was determining for each animal before treatment and after 2 minutes (time determine according to pilot study) of aspirin (gel, cream, ointment) topical application on fore and back paw starting by 1% reliant on previous study. The change of increase dose and decrease dose is 0.5%. The analgesic EC_{50} for aspirin (gel, cream and ointment) were determined according to (19).

Evaluation of (EC_{50}) of aspirin nanoparticles (gel, cream and ointment)

Seventeen mice were used in our study to evaluate the median analgesic concentration (EC_{50}) of aspirin nanoparticles (gel, cream and ointment), by using up and down method (19). The pain threshold was determining for each animal before treatment and after two minutes of aspirin nanoparticles gel topical application on fore and back paw starting by 1%. The change of the dose increasing and decreasing were 0.25% for aspirin nanoparticles (gel, cream and ointment). The EC_{50} for aspirin nanoparticle was determined according to (19).

Results

Aspirin gel

The Median Effective Concentration (EC_{50}) of aspirin gel after topical applied in mice is 0.848 % (Table 1).

Aspirin cream

The median effective concentration (EC_{50}) of aspirin cream topical application in mice is 0.958% (Table 2).

Aspirin ointment

The median effective doses (EC_{50}) of aspirin ointment topical application in mice is 0.958% (Table 3).

Aspirin nanoparticles gel

The median effective concentration (EC_{50}) of aspirin nanoparticles gel topical application in mice is 0.72% (Table 4).

Table 1: Evaluation of median analgesic concentration (EC_{50}) of aspirin gel

Variable	Result
EC_{50}	0.848% topical
Range of the concentration used	0.5-1.5 % topical
Initial concentration	1% topical
Last concentration	1% topical
Animal number	5 (XOOXX)
Concentration change	0.5 % topical

Table 2: Evaluation of median analgesic concentration (EC_{50}) of aspirin cream

Variable	Result
EC_{50}	0.958% topical
Range of the concentration used	0.5 - 1 % topical
Initial concentration	1 % topical
Last concentration	1 % topical
Animal number	5 (XOXOO)
Concentration change	0.5 % topical

Table 3: Evaluation of median analgesic concentration (EC_{50}) of aspirin ointment

Variable	Result
EC_{50}	1.00 % topical
Range of the concentration used	0.5 - 1 % topical
Initial concentration	1 % topical
Last concentration	1 % topical
Animal number	5 (XOXOO)
Concentration change	0.5 % topical

Table 4: evaluation of median analgesic concentration (EC_{50}) of aspirin nanoparticles gel

Variable	Result
EC_{50}	0.72 % topical
Range of the concentration used	0.5 - 1 % topical
Initial concentration	1 % topical
Last concentration	0.5 % topical
Animal number	5 (XOXXO)
Concentration change	0.25 % topical

Aspirin nanoparticles cream

The median effective concentration (EC_{50}) of aspirin nanoparticles cream topical application in mice is 0.657% (Table 5).

Aspirin nanoparticles ointment

The median effective concentration (EC_{50}) of aspirin nanoparticles ointment topical application in mice 0.657% (Table 6).

Table 5: Evaluation of median analgesic concentration (EC_{50}) of aspirin nanoparticles cream

Variable	Result
EC_{50}	0.657 % topical
Range of the concentration used	0.5 - 1 % topical
Initial concentration	1 % topical
Last concentration	0.75 % topical
Animal number	6 (XXOOXX)
Concentration change	0.5 % topical

Table 6: Evaluation of median analgesic concentration (EC_{50}) of aspirin nanoparticles ointment

Variable	Result
EC_{50}	0.701 % topical
Range of the concentration used	0.5 - 1 % topical
Initial concentration	1 % topical
Last concentration	1 % topical
Animal number	6(XXOOXO)
Concentration change	0.25 % topical

Discussion

Oral analgesics are usually given for treatment of pain (acute and chronic), aspirin which is one of the most common one although it is old and in expensive drug it usually showed several adverse effects even with small dose like gastrointestinal disorder, urticaria, asthma exacerbation, angioedema, in addition to more sever and rare adverse effects like thrombocytopenia, interstitial nephritis, and prolongation of prothrombine time (20,21).

Topical analgesic provides a promising route in giving the same analgesic effects provided by oral analgesic but with negligible adverse effects (22). In our study we tried to use a transdermal delivery system (by formulation of aspirin gel, ointment and cream in normal and nanostructure) as alternative route of administration which bypass the gastrointestinal tract in addition it is more safe and convenient for patients in prolong use. According to the result of our study better analgesic effects was demonstrated with aspirin gel (EC_{50}=0.848%) compared with equal analgesic effects of aspirin cream and ointment (EC_{50}=0.958%). this is disagreement with the study of Ravindra *et al* who showed that ointment give a better result (18). Aspirin delivery in our study was greater from gel formulation than from ointment and cream which may demonstrate that physical factor in the donor vehicle are more effective in controlling the release of the active ingredient from gel than are chemical factors (1).

Aspirin is slightly water soluble so it has a law affinity to the gel formulation, so it partitions to large extent into the lipophilic situation of the membrane of mice skin, the rapid flux rate seen in this process are concentration gradient dependence (23). on the contrary its suggested that ointment has relatively smaller affinity for the skin mice than for gel and therefore showed a lower partition and flux parameter in addition to lower flux rate also observed (24). The cream is lipophilic hydrophilic blend so the partition between two phases so slower flux rate are showed compared with ointment and gel (25)

The results obtained from this study showed that nanoparticles produce a better antinociceptive effects than normal size particles in all the three topical form (gel, ointment, and cream), this partially in agreement with study of Subramanian *et al*, who showed that nano emulsion preparation of aspirin will enhance the anti-inflammatory effects of aspirin in mouse model (26), this may be due to increase bioavailability of aspirin in nanostructure as it reported by other studies (27,28). The other evidence which support this is the increased bioavailability of paclitaxel transdermal system showed by pharmacokinetic analysis (29).

Conclusion

This study formulates a transdermal delivery system (topical form) for aspirin (gel, ointment and cream) and demonstrate that the analgesic effect of gel is better compared with ointment and cream in addition the antinociceptive effects of aspirin are enhance by Nano formulation of all three vehicles in mice model.

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Conflict of interest

Authors states that she has no known rival monetary attention or private dealings that could affect the work of this manuscript

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مقارنة التأثير المسكن للأسبرين والجسيمات النانوية للأسبرين في التراكيب شبه الصلبة في الفئران

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الخلاصة

يستخدم الأسبرين بشكل شائع في الطب كمسكن ومضاد للحرارة ومضاد للالتهاب، والإعطاء الفموي هو الأكثر شيوعاً، ونتيجة لذلك سوف ينتج آثاراً ضارة مختلفة مثل القرحة المعدية واعتلال الكلية ونقص الصفيحات حتى مع جرعة علاجية صغيرة، لذلك فإن الاستخدام الموضعي هو الطريقة البديلة والمفضلة مع الحد الأدنى من الآثار الجانبية والاستجابة العلاجية الفعالة. لذلك، في هذه الدراسة، تم التحقق مما إذا كانت الخاصية المضادة للمسببات للأسبرين ستعزز إذا استخدمت الأسبرين كجزيئات نانوية بعد تحضيره بعدة أشكال (هلام، كريم ومرهم). اثنان وثلاثون فأرا ذكرا صحيا تزن ٣٠-٣٥ غرام استخدمت في الدراسة الحالية. تم تقسيم الحيوانات عشوائيا وتم علاج كل فأر موضعيا. تم تحضير تراكيز الأسبرين باستخدام الجل والكريم والمرهم كوسيط ناقل واستخدموا موضعياً على القدم الأمامية والخلفية للحيوانات التجريبية. تم تحفيز الألم عن طريق اختبار الصفيحة الساخنة لتقييم مدة الألم. تم تسجيل الوقت من وضع الحيوان على الصفيحة إلى أن يقفز أو يلعق أقدامه وسجل كفترة استجابة. أظهرت النتائج أن التركيز الوسطي للتأثير المسكن للأسبرين بشكل (هلام، كريم ومرهم) كان ٠,٨٤٨، ٠,٩٥٨، ١,٠٠% على التوالي بينما انخفض التركيز الوسطي للتأثير المسكن عند استخدام جزيئات النانوللاسبرين بشكل (هلام، كريم ومرهم) إلى ٠,٧٢، ٠,٦٥٧ و ٠,٧٠١٪ على التوالي. نستنتج أن لاستخدام الأسبرين عبر الجلد تأثيرات علاجية فعالة مضادة للألم في الفئران على الرغم من أن تحضير الأسبرين مع الهلام ينتج استجابة أفضل يتبعها الأسبرين مع الكريم، ثم الأسبرين مع المرهم ويرجع ذلك بسبب الاختلاف بالخصائص الحركية الدوائية بين (الهلام، الكريم والمرهم). كما أن تحضير الجسيمات النانوية ينتج استجابة فائقة في جميع الأشكال سوآءا أكان النانواسبرين محضر بشكل هلام، كريم أو مرهم.

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