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The Toxicity and Therapeutic Efficacy of Mefenamic Acid and its Hydroxyethyl Ester in Mice: *In Vivo* Comparative Study: A promising Drug Derivative

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ABSTRACT

Background: Hydroxyethyl Ester of Mefenamic acid (HEMA), which is an available derivative of mefenamic acid (MFA) in the literature, was shown to exert a strong resistance to enzymatic hydrolysis in various buffer solutions as well as in the plasma. However, there are no studies yet that investigate the biological effects of HEMA as a possible active drug in-vivo. This study provides an in-vivo investigation of the efficacy and toxicity of HEMA in comparison to those of a related drug, MFA, that has a similar chemical structure.

Methods: Acute toxicity evaluations were conducted in various groups of mice following administration of high equimolar doses of HEMA and MFA and were measured at various parameters including the percentage of catalepsy, seizure score, percentage of clonic-tonic seizure and death, grimace scale score (GSS) and locomotor activity. In addition, the anti-inflammatory and anti-nociceptive effects of HEMA were evaluated in the carrageenan-induced paw edema test and acetic acid-induced writhing test, respectively.

Results: The findings of this study revealed that the percentage of catalepsy, clonic-tonic seizure and death as well as seizure and grimace scale scores were lower in mice treated with HEMA than those treated with equimolar doses of MFA. In addition, treatment with HEMA caused a comparable anti-inflammatory activity in the carrageenan-induced paw edema test and a significantly ($p<0.05$) higher anti-nociceptive effect in the acetic acid-induced writhing test than that of MFA.

Conclusion: Results obtained from this study may indicate that HEMA has superior therapeutic advantages for the management of acute and inflammatory events with a less potential risk of neuromuscular adverse effects.

Keywords: Mefenamic acid, Ester, Seizure, catalepsy, antinociceptive.

1. INTRODUCTION

Structural modification of existing drugs is a well-established approach in drug design and development [1,2]. This approach involves the alteration, addition, or removal of functional groups in drug structure that could help to produce new drug derivatives with improved functional properties [3]. The main objective of such an approach is to enhance the clinical outcomes in term of enhancing the therapeutic effects and reducing drug toxicities [3].

Mefenamic acid (MFA),which is a common non-steroidal anti-inflammatory drug (NSAID), is a highly lipophilic agent that is rapidly absorbed across the gastrointestinal tract and distributed throughout tissue fluids [4]. It can cross the blood-brain barrier and interacts with various molecular targets in the brain including the cerebral cortex and hippocampus[5,6]. From the clinical point of view, the presence of MFA in the plasma,

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above the minimum effective level, is associated with a high risk of muscle twitching that ends by grand mal convulsion in 38% of the patients (7,8). A couple of comparative investigations also revealed that MFA treatment in humans is implicated in a large number of central nervous system (CNS) convulsions compared to other NSAIDs (9). On the other hand, a line of emerging evidence has recently shown that MFA exerts multiple biological effects that potentiate its use in a wide range of clinical applications (10). For example, MFA has emerged as a potent drug to treat schistosomiasis (11). In addition, MFA is thought to reduce depressive symptoms and improve cognitive impairment disorders in the rodents (12,13). MFA use was also reported to be effective as adjuvant therapy in the treatment of castration-resistant prostate cancer in a randomized control trial (14). These together may indicate that MFA is a promising therapeutic for various diseases, but its neuromuscular adverse effects may considerably offset its therapeutic advantages. In line of these, the present study was conducted with the assumption that reducing the neuromuscular adverse effects of MFA using the structural modification approach may constitute an inflection point on its clinical development. Therefore, there is a constant need for developing various analogs of MFA that inherit great therapeutic benefits and pose minor adverse effects on the biological systems.

Hydroxyethyl ester of MFA (HEMA) is an analog of MFA that has been available in the literature since 1979. Structurally, the hydroxyethyl moiety of HEMA binds via an ester bond to the carboxylic acid group of MFA, which is responsible for the development of seizure in MFA treatment (15). The ester bond of HEMA showed strong resistance to enzymatic degradation and exhibited high stability in the plasma and various buffer solutions (16,17). It is therefore expected that HEMA has less toxicity on the neuromuscular system than MFA while its therapeutic effects remain to be answered. Based on the findings of previous studies, there is a contradiction in the research regarding the functional role of the carboxylic group as an essential pharmacophore in various NSAIDs. However, the recent trend of the research may incline to refute the functional relationship between the carboxylic acid group and the therapeutic response (18). In light of this, the present study provides a comparative investigation on neuromuscular toxicity, antinociceptive activity, and anti-inflammatory effect between HEMA and MFA.

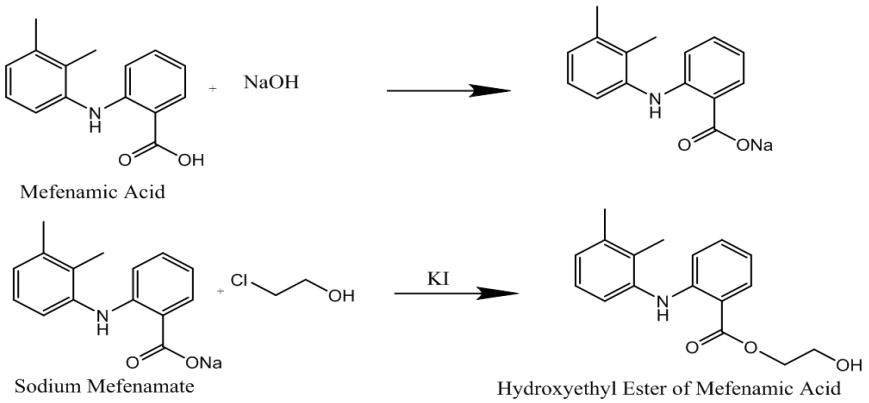
2. Chemicals and methods

2.1. Chemicals and instruments

Mefenamic acid, dimethyl sulfoxide, 2-chloroethanol and carrageenan were purchased from Sigma, US. Acetic acid and formalin were obtained from BDH, UK. The ^{1}H- NMR of HEMA was recorded on a Bruker 500MHz spectromter (Bruker DPX-500) and was analyzed using TopSin 3.2 Windows software. Activity cage (Ugo Basile, Italy) was used for measurement of locomotor activity in mice.

2.2. Synthesis of HEMA

Synthesis of HEMA was conducted according to a procedure described previously (16). A mixture of the sodium salt of mefenamic acid (3.0 g, 0.011 mol); in dry DMF (15 ml) with potassium iodide (0.18 g, 0.0012 mol) was stirred for 5-10 minutes to obtain a suspension, and then bromoethanol (1.5 g, 0.012 mol) was added in a dropwise manner. The reaction mixture was stirred for 7 hr with heat at 70°C. After the reaction was completed, the reaction mixture was treated with 30 ml ethyl acetate to form a precipitate, which was filtered on a Whatman ® cellulose filter paper (Grade 40 Ashless Filter Paper 150 mm). The filtrate was treated with 15 ml of aqueous solution of sodium thiosulfate (5% w/v) for 3 times. After evaporation of the organic solvent, the obtained oil was poured into a beaker containing ice water to produce a solid precipitate. The precipitate was filtered by a Buchner funnel and recrystallized by ethanol to yield 2.4 g. melting point 80°C; IR 1680 cm-1 (ester),1HNMR (CD3CN), d, 2.1(s, 3H, CH3), 2.3 (s, 3H, CH3), 3.8 (t,2H, CH2), 4.3 (t, 2H, CH2), 6.6-8 (m, 7 H aromatic), 9.2 (s, 1H, NH). Scheme 1 shows synthesis reaction of HEMA.



Scheme 1. General scheme for HEMA synthesis

2.3. Animals

Male Swiss albino mice weighing between 21-33 g were used in various experiments. These mice were born and reared in the animal facility of Isra University, Jordan. All animals were subjected to controlled conditions (22-25 C, 67-79% humidity and 12/12-hour light/dark cycle), and were housed in polypropylene cages (30*22*16 cm^3) provided with a free access to pelleted diet and tap water. All experiments were conducted between 9am-5pm with the permission granted by Scientific Research Committee of Isra University (2019/2018/17-174). All procedures of animal use and handling were conducted according to the laboratory guidelines of animal care (19).

2.4. Toxicological evaluations

Acute toxicity study was conducted according to procedures described by OECD guidelines (OECD Guidelines, 2010). The main objective of this study is to evaluate the acute adverse effects of HEMA in comparison to those of MFA. The adverse effects were evaluated across various measuring parameters using various experimental sessions. For each session, mice were distributed into three groups (n=6/each group) as follows:

Group I (Vehicle control): Each mouse of this group received a single non-toxic dose of drug vehicle (DMSO, 1.2 mL/kg), via the intraperitoneal (i.p) route. The dose was selected based on a previous study (20)

Groups II (Positive control): Each member of this group received a single dose of MFA via the i.p. route. Different doses were used in various experimental sessions for induction of the adverse effects via the i.p. route. All doses were selected based on a previous study (15).

Group III (test group): Mice of this group were received HEMA at equimolar dose to MFA.

Details of experimental procedures were presented in subsections as stated below.

2.4.1. Assessment of acute distress after drug dosage

Acute distress that is associated with HEMA side effects was evaluated in comparison to the distress produced by MFA treatment (40 mg/kg) using the mouse grimace scale (Fig.1). At 30 minutes after the treatments, mice were transferred individually in a glass box (30*30*30 cm^3) and observed for 15 minutes. During the observation, a series of photos (n=60 photos) was taken randomly by pointing the camera directly at the mouse's head. These photos were cropped to create a clear focus on the mouse's face and reduce the risk of bias due to body posture. The feature of the mouse's face in these photos was analyzed across five action units: orbital tightening, nose bulge, check bulge, ear position and whisker change (Fig.1). Each action unit was taken a value of 0 (indicates

the action unit was absent in all photos), 1 (indicates the action unit was present in at least one photo with a mild feature) or 2 (indicates the action unit was obvious). The sum of scores was taken in each mouse and the totals were averaged in each group.

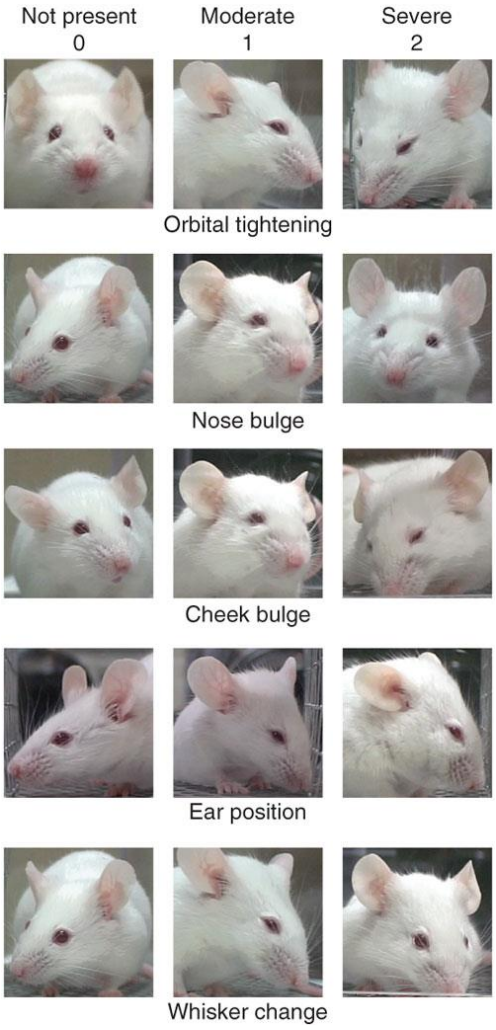


Fig.1. Grimace scoring scale for assessment of distress following drug dosage

2.4.2. Assessment of catalepsy

Catalepsy was evaluated in mice treated with HEMA in comparison to those treated with MFA at 40 mg/kg. After 30 minutes of the treatments, mice were placed individually in an open arena and were gently forced to place their front paws on a 5-cm high wooden bar. The mouse which showed no resistance against the bar for 20 seconds were considered as cataleptic mice. The percentage of catalepsy was calculated in each group and used as index for measuring muscle spasm in mice.

2.4.3. Assessment of seizure

Seizure was evaluated in mice treated with HEMA in comparison to those treated with MFA at 80 mg/kg. Immediately after treatments, mice were transferred to a

glass beaker for observation. The percentage of seizure was calculated in each group and seizure score was estimated using the scoring scale presented in Table 1.

Table 1. Scoring scale of seizure in mouse

Score	Observation
1	Akinesia (loss of voluntary movement) 
2	Head nodding (head movement in alternating up and down arcs along the vertical cervical axis), partial myoclonus 
3	Contentious whole-body myoclonus 
4	Rearing tonic seizure (The mouse shows standing up position with clonic bilateral forelimb movements) 
5	Tonic-clonic seizure, wild rush and jumping 

2.4.4. Assessment of death

Death was evaluated in mice treated with HEMA in comparison to those treated with MFA at 80 mg/kg. The number of deaths over 14 days post-treatment was counted in each group and the mortality percentage was then calculated.

2.4.5. Assessment of locomotor activity

The locomotor activity of mice treated with HEMA was evaluated in comparison to that seen in mice injected with MFA (40 mg/kg) using the actophotometer model. After 30 minutes of treatments, mice were placed individually for 10 minutes in the actophotometer for evaluation of the locomotor activity. The number of light beam interruptions that resulted from the horizontal movement of mice was counted and was used as index for the locomotor activity.

2.5. Therapeutic evaluations

The therapeutic effects of HEMA was evaluated using various in-vivo experiments. For each experiment, mice were distributed to three groups and treated as follows:

Group I (Vehicle control): Each mouse of this group received drug vehicle (DMSO, 1.2 mL/kg), via the intraperitoneal (i.p) route.

Groups II (Positive control): Each member of this

group received intraperitoneal dose of MFA (20 mg/kg)

Group III (test group): Mice of this group were injected intraperitoneally with HEMA at an equimolar dose to MFA.

3.5.1. Assessment of anti-inflammatory effect

The anti-inflammatory effect of HEMA was evaluated in comparison to that of MFA using the carrageenan-induced paw edema test as described previously (21). After 30 minutes of drug treatment, mice were challenged with sub-planter injection of carrageenan solution (1%, 0.1 mL) in the right hindpaw . Paw thickness was measured immediately (at time 0) and at 1,2,3 and 4 hours after carrageenan injection using a digital caliper. Reduction in paw thickness in comparison to those of vehicle control was used as index of anti-inflammatory effect.

3.5.2. Assessment of anti-nociceptive effect

3.5.2.1. Acetic acid-induced visceral pain test

The acetic acid-induced writhing test was used to evaluate the anti-nociceptive effect of HEMA according to a procedure described previously (22,23). After 30 minutes of drug treatment, each mouse was injected intraperitoneally with 0.6% of acetic acid solution and was then placed in transparent Perspex box for observation. Following a 5-minutes lag period, the writhing responses which characterized by limb extension, tail erection and body elongation were cumulatively counted over a period of 30 minutes. Reduction of writhing effects in comparison to vehicle mice was used as index of anti-nociceptive effect.

3.5.2.2. Formalin -induced paw licking test

The anti-nociceptive effect of HEMA against formalin-induced paw licking was evaluated in comparison to that of MFA using a method described previously (24). After 30 minutes of drug treatment, mice received sub-plantar injection of formalin into right hind paw for induction of pain that characterized by repeating paw licking behavior. The time spent for licking the paw was measured during the initial phase (0-10 minutes) and during the late phase (15-30 minutes). Reduction in the licking time was used as index for anti-inflammatory effects of drugs.

2.6. Statistical analysis

Data obtained from different animal experimentations are presented as an average of duplicate trial ± standard deviation (SD). The statistical difference between groups was calculated by one-way analysis of variance (ANOVA) followed by post hoc Tukey's test using Statistical Package for the Social Sciences (SPSS) program, version 22.0 (IBM). A probability value of less than 0.05 ($p<0.05$) was set as a significant difference.

3. Results

3.1. Acute toxicity test

Data obtained from the acute toxicity study revealed that treatment with MFA, via the intraperitoneal route, was capable to induce multiple side effects in a dose-dependent manner. At 40 mg/kg, mice showed a prominent change in grimace score with a marked decrease in the locomotor activity that was associated with muscles spasm and catalepsy. On the otherhand, treatment with 80 mg/kg induced tonic-clonic seizures that are usually ended up with death. In this study, HEMA was synthesized as a modified structure from MFA aiming to reduce the acute adverse effects, particularly on the nervous system. The results showed that mice treated with HEMA had significantly lower grimace scores than those received MFA (Fig.2), which were manifested by a significant decrease in the severity of eyelid close with normal ear position and check and nose bulges that were almost similar to vehicle control. In addition, mice treated with HEMA showed a lower decrease in locomotor activity as compared to mice treated with MFA (Fig.3). The incidence of catalepsy and seizure was less evidenced in mice injected with HEMA (Fig.4 and Fig.5 respectively). The severity of seizure was also reduced in mice treated with HEMA as they exhibited non-fatal head-nodding whereas mice treated with MFA showed tonic-clonic seizures (Fig.6). Moreover, the number of deaths during 14 days period was higher in the group that received MFA than that treated with HEMA (Fig.7).

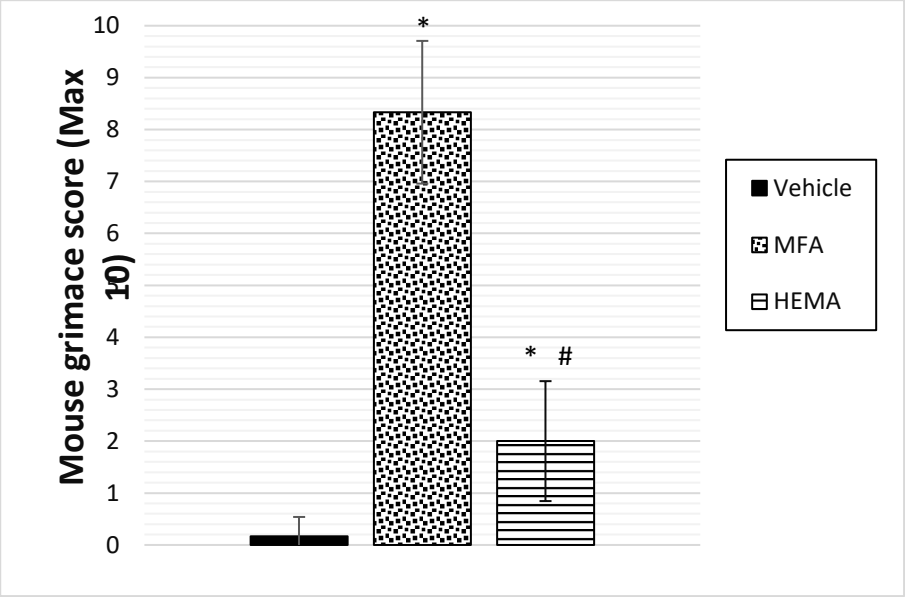


Fig.2. Mean of grimace score in mice received vehicle, MFA and HEMA. (*) Indicates significant ($p<0.05$) difference from vehicle group. (#) Indicates significant ($p<0.05$) difference from MFA group.

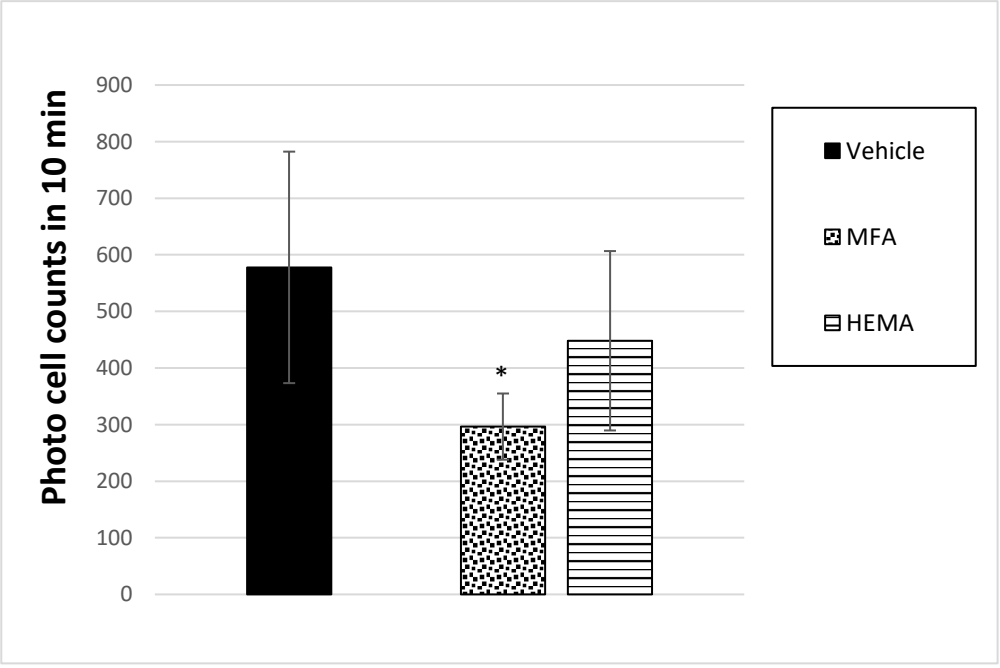


Fig.3. Mean of photo cell counts in mice received vehicle, MFA and HEMA. (*) Indicates significant ($p<0.05$) difference from vehicle group. Note that there is a significant decrease in locomotor activity in the group treated with MFA but not group treated with HEMA

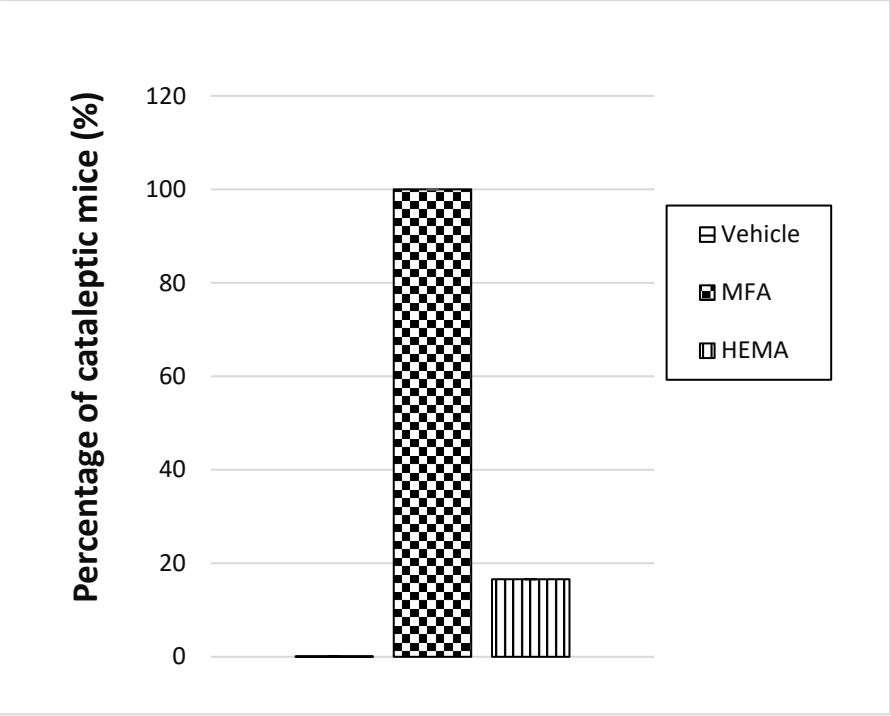


Fig.4. Percentage of cataleptic mice received vehicle, MFA and HEMA

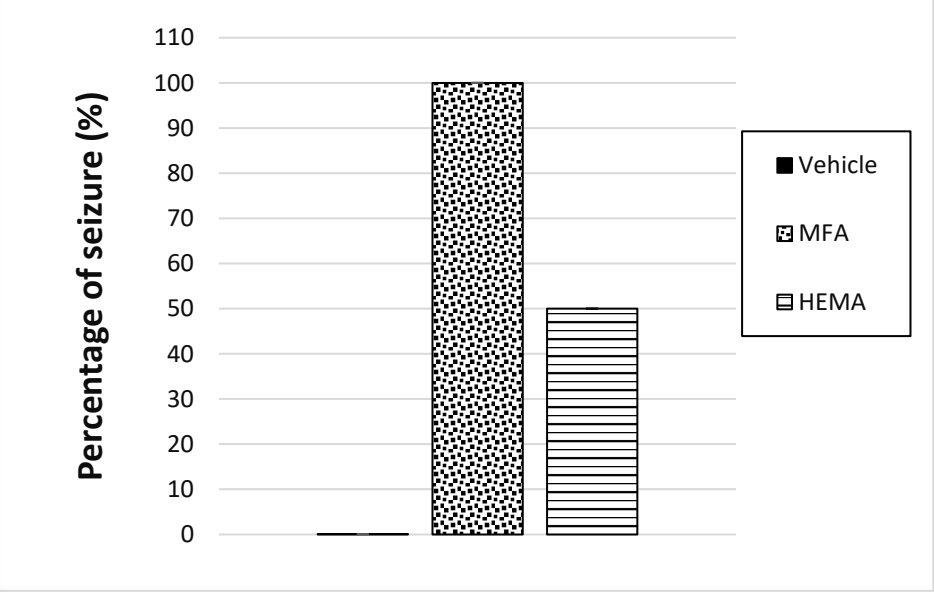


Fig.5. Percentage of seizure in mice received vehicle, MFA and HEMA

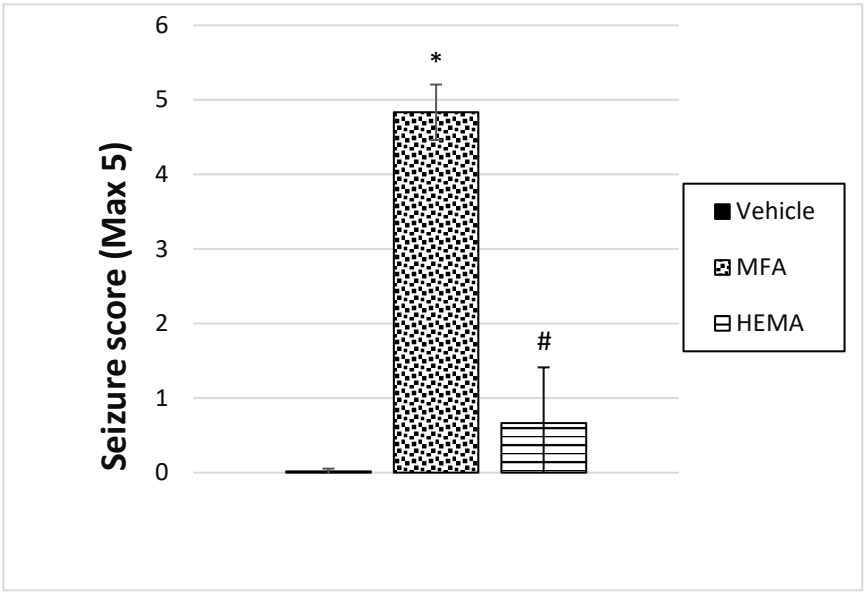


Fig.6. Mean of seizure scores in mice received vehicle, MFA and HEMA. (*) Indicates significant ($p<0.05$) difference from vehicle group. (#) Indicates significant ($p<0.05$) difference from MFA group.

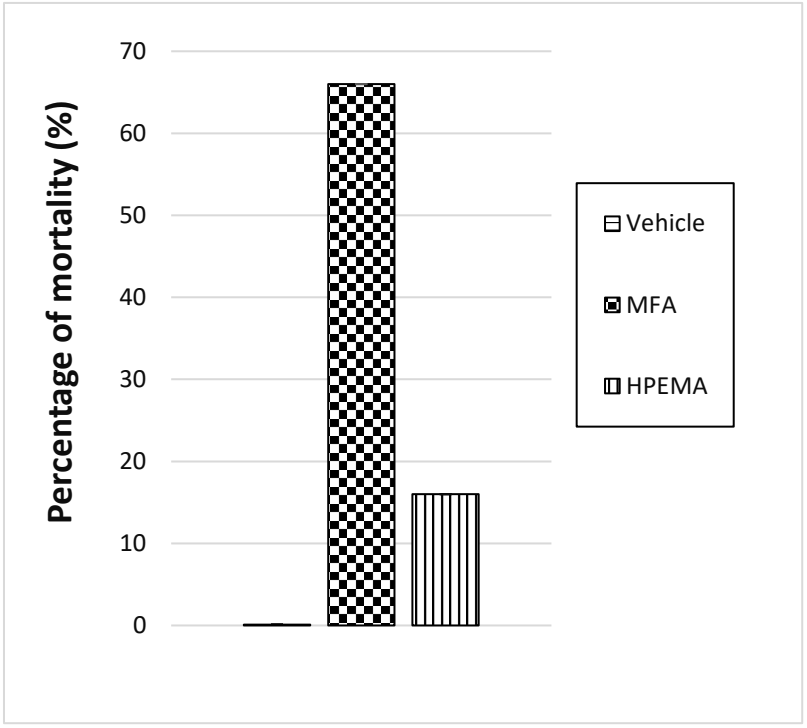


Fig.7. Percentage of mortality in mice received vehicle, MFA and HEMA

3.2. Carrageenan-induced hind paw edema test

Measurements of hind paws thickness following carrageenan injection showed a maximum increase of paw edema at 3-4 hours post injection. Pretreatment with MFA and HEMA caused significant decrease in paw thickness as compared to vehicle effect at 3 hours following carrageenan injection (Fig.8). Although mice treated with MFA showed higher decrease of paw thickness than group treated with HEMA at various time points, the statistical analysis didn't find any significant difference between these groups (Fig.8).

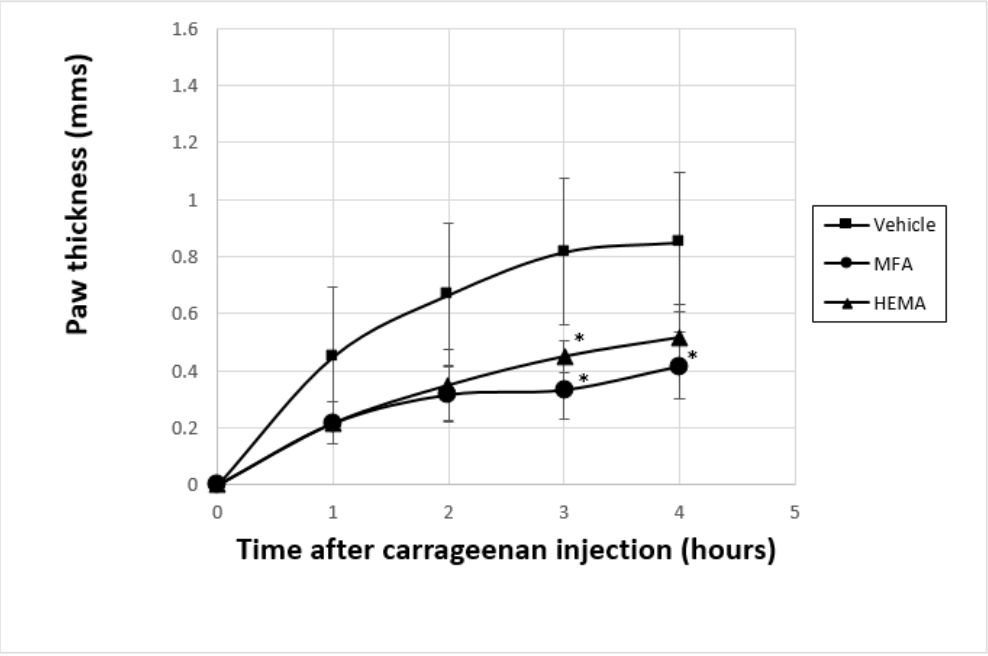


Fig.8. Mean of paw thickness in mice received vehicle, MFA and HEMA. (*) Indicates significant ($p<0.05$) difference from vehicle group.

3.3. Acetic acid-induced writhing response test

Observation of mice following acetic acid injection showed writhing responses that were represe nted by multiple attack of hind limbs extension and waist twisting. However, pretreatment with MFA and HEMA significantly reduced the number of writhing responses as compared to the effect of vehicle (Fig.9). In addition, the reduction of writhing effects was significantly lowered in group treated with HEMA than group treated with MFA (Fig.9).

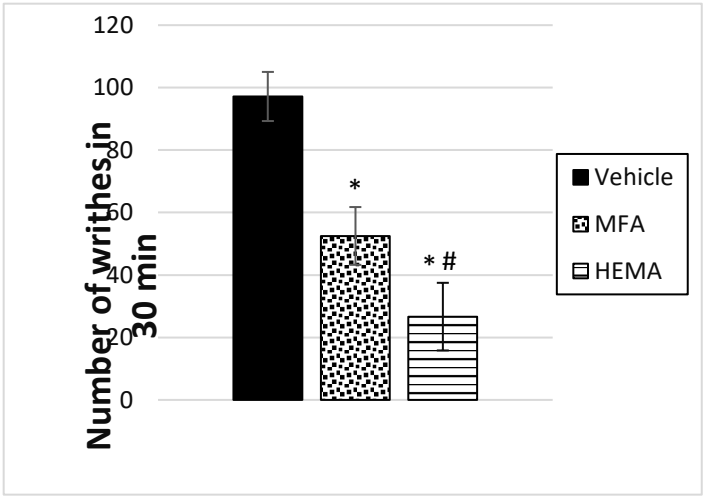


Fig.9. Mean of writhing effect numbers in mice received vehicle, MFA and HEMA. (*) Indicates significant ($p<0.05$) difference from vehicle group. (#) Indicates significant ($p<0.05$) difference from MFA group.

3.4. Formalin-induced hind paw licking test

Observation of mice following formalin injection showed paw licking behaviors at two distinctive phases. Pretreatment with MFA and HEMA significantly reduced the time spent for paw licking during the late phase only as compared to the effect of vehicle (Fig.10). No significant difference in paw licking time between group treated with HEMA and group treated with MFA.

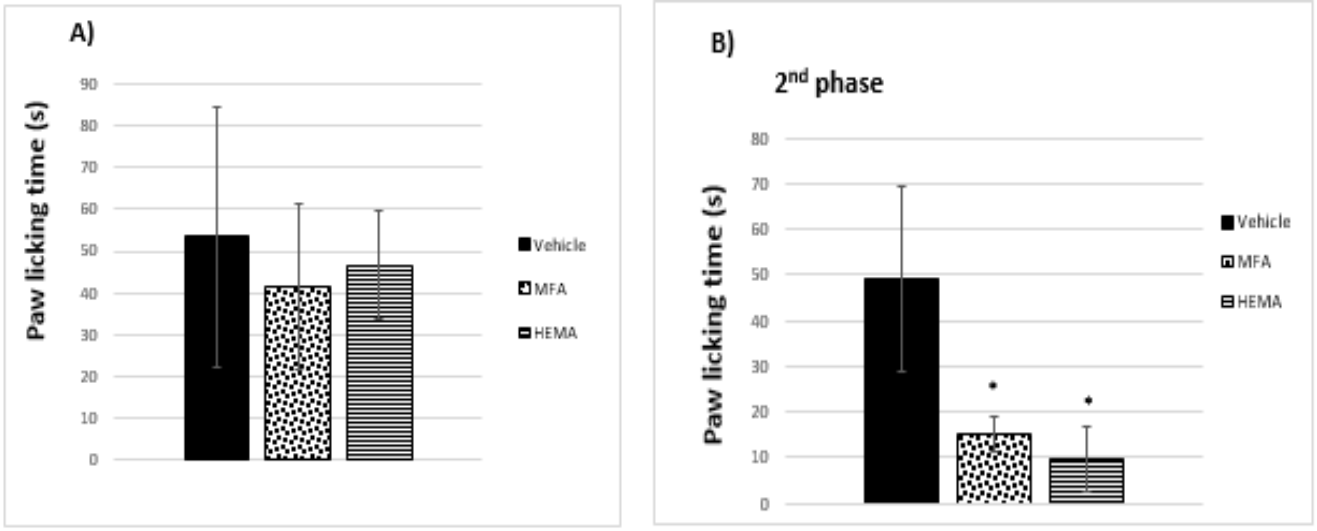


Fig.10. Mean of paw licking time in mice received vehicle, MFA and HEMA. Subfigure (A) represents the first phase of the formalin induced licking test. Subfigure (B) represents the second phase of the formalin induced licking test. (*) Indicates significant ($p<0.05$) difference from vehicle group.

4. Discussion

Chemical modifications on drugs is a common pharmaceutical approach that has a great potential to enhance the pharmacodynamics and pharmacokinetics and create novel drugs (25,26). For example, many efforts have been made for enhancing the therapeutic activity of NSAIDs and reducing their toxicity by developing chemical derivatives or using prodrug approach (27). Concerning MFA, which is a potent analgesic and antipyretic drug, the CNS toxicity manifested by serious convulsions is common in a clinical practice after ingestion of high doses (8,9). Therefore, the recent trend in MFA development is to reduce its CNS toxicity by masking the carboxylic acid moiety. A previous study demonstrated that binding MFA with alpha-tocopherol reduced the CNS toxicity by increasing the latency to the convulsions (15). In this study, the potential risk of CNS toxicity of HEMA was evaluated in comparison to that of MFA. The finding revealed that mice treated with HEMA showed less seizure percentage, seizure score, catalepsy percentage and mortality percentage. These results together indicate that treatment with HEMA has less potential to cause CNS adverse effects than MFA. The neurobiology of CNS convulsion, catalepsy and motor dysfunction is thought to be caused by disruptions in the GABAergic, glutamatergic, and dopaminergic systems (28–32). However, a line of molecular research revealed that MFA is capable to interact with GABAergic function in a dose-dependent manner. (33,34). In this regard, the ability of HEMA to prevent CNS toxicity is assumed to be attributable to pharmacokinetic changes or a change in drug release profile that reduce the risk of GABAergic disruption.

Paw edema thickness of mice, following a sub-planter injection of carrageenan, has been frequently used in experimental works as an index for development of acute topical inflammation in paw tissues (35,36). It was well identified that development of edema due to carrageenan injection has a biphasic nature (37). The initial phase is attributed basically to histamine and serotonin secretion due direct tissue damage following carrageenan injection. On the other hand, the late phase, which start at 3-6 hours after carrageenan injection, involves a massive production of prostaglandin that works as a potent inflammatory and nociceptive mediator. In this study, mice treated with HEMA showed significant reduction in paw thickness at the second phase only when compared with vehicle mice. These results may indicate that treatment with HEMA produced a considerable anti-inflammatory effect in vivo that may relate to a possible inhibitory effect on prostaglandins synthesis. The results also revealed that the anti-inflammatory of HEMA in carrageenan-induced licking test was statistically comparable to that of MFA, indicating that HEMA attained the anti-inflammatory properties of MFA.

Pain is abnormal sensory perception characterized by uncomfortable sensation that works as alarming signal to harmful stimuli (38). It constitutes a fundamental symptom in various diseases and pathological conditions including acute and chronic inflammations (39). It has been identified by various health care organizations as one of the most disabling disorders that reduces the quality of life and interferes with social activities(40,41). The pathogenesis of pain has a complex nature that involves dysregulation of various molecular and biochemical pathways in the peripheral and central nervous system (42). Treatment of pain with opioid and non-opioid analgesics has been reported with suboptimal therapeutic effects or associated with serious adverse effects(43,44). Therefore, it is always coveted to develop safer and effective drugs for the use in the pharmaceutical and clinical practice. In this study, HEMA was evaluated, for the first time, as possible analgesic drug in acetic acid induced writhing test and formalin induced paw licking test in mice.

Acetic acid induced writhing test is a well-accepted and validated method for evaluating of anti-nociceptive activity in mice (45,46). Injection of diluted acetic acid in the abdominal cavity renders mice to exhibit a pain response that basically manifested by arching of back, limb extension and tail

erection that together known as "writhing response " (46). A line of evidence revealed that common analgesics were effective in reducing writhing response following acetic acid injection (45,47). In this study, HEMA caused significantly stronger inhibition that produced by the vehicle and MFA. This may indicate that HEMA exerts a sufficient analgesic property for the clinical use.

Formalin test demonstrates a biphasic response of paw licking in mice following formalin injection (24,48). The initial phase, which also known as neurogenic phase, starts immediately after formalin injection and last for approximately 5 minutes. The second phase (inflammatory response) occurs during 15-30 minutes following formalin injection. It is reported that active drugs act differently on both phases based on its mechanism of actions (49). Centrally acting drugs were found to inhibit both phases, whereas most NSAIDs tends to inhibit the second phase only. Therefore, it is well accepted that generation of pain during the second phase may occur basically due to a serial of inflammatory events following paw tissues damage after formalin injection. In this study, HEMA caused a significant inhibition in the second phase which was compared to that produced by MFA. These results may indicate that the analgesic effect of HEMA owes to it is anti-inflammatory properties in-vivo.

5. Conclusion

Binding the carboxylic group of MFA with hydroxyethyl moiety reduced the acute CNS toxicity without delaying or reducing the therapeutic effects. This may indicate that the free carboxylic group of MFA implicates in CNS toxicity but it does not serve as an essential pharmacophore for the anti-inflammatory and anti-nociceptive effects. In addition, the higher anti-nociceptive effect of HEMA may indicate a different pharmacological profile from MFA. On the other hand, the lower CNS toxicity of HEMA increases its potential use for the parenteral application. These together may suggest that HEMA serves as an active derivative of MFA that can be safely used for treatment of acute pain and inflammatory events in future. However, further studies are needed to investigate the chronic toxicity of HEMA following repeated dose administration via different routes of administration (i.e oral and intramuscular routes). In addition, the questions about its pharmacokinetic and metabolic profiles need to be answered.

6. Acknowledgment

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REFERENCES

1. Kai-chuan Y, Li Y, Jia-jian L, Qiang L. Research on structural modification of cephalosporinal antibiotics [J]. World Notes Antibiot. 2009;4.
2. Cheng XC, Liu XY, Xu WF. Recent advances in the structural modification of ligustrazine: cerebro-and cardiovascular activity of ligustrazine derivatives. Drugs Future. 2005;30(10):1059–65.
3. Anderson AC. The process of structure-based drug design. Chem Biol. 2003;10(9):787–97.
4. QAMAR S, IRFAN N, AHMAD M, JAMSHAID M, MUZAFFAR NA. Effect of dehydration on the pharmacokinetics of mefenamic acid. Turkish J Med Sci. 1999;29(2):101–4.
5. Khansari PS, Halliwell RF. Mechanisms underlying neuroprotection by the NSAID mefenamic acid in an experimental model of stroke. Front Neurosci. 2019;13:64.
6. Hernandez A, Paeile C, Perez H, Ruiz S. Cortical facilitatory effect of mefenamic acid. Arch Int Pharmacodyn Ther. 1980;244(1):100–6.
7. Robson RH, Balali M, Critchley J, Proudfoot AT, Prescott L. Mefenamic acid poisoning and epilepsy. Br Med J. 1979;2(6202):1438.
8. Balali-Mood M, Proudfoot AT, Critchley JAJH, Prescott LF. MEFENAMIC ACID OVERDOSAGE. Lancet [Internet]. 1981;317(8234):1354–6. Available from:

https://www.sciencedirect.com/science/article/pii/S0140673681925289

9. Kamour A, Crichton S, Cooper G, Lupton DJ, Eddleston M, Vale JA, et al. Central nervous system toxicity of mefenamic acid overdose compared with other NSAIDs: an analysis of cases reported to the United Kingdom National Poisons Information Service. Br J Clin Pharmacol. 2017;83(4):855–62.
10. Cimolai N. The potential and promise of mefenamic acid. Expert Rev Clin Pharmacol. 2013;6(3):289–305.
11. Lago EM, Silva MP, Queiroz TG, Mazloum SF, Rodrigues VC, Carnaúba PU, et al. Phenotypic screening of nonsteroidal anti-inflammatory drugs identified mefenamic acid as a drug for the treatment of schistosomiasis. EBioMedicine. 2019;43:370–9.
12. Feng X, Fan Y, Chung CY. Mefenamic acid can attenuate depressive symptoms by suppressing microglia activation induced upon chronic stress. Brain Res. 2020;1740:146846.
13. Joo Y, Kim H-S, Woo R-S, Park CH, Shin K-Y, Lee J-P, et al. Mefenamic acid shows neuroprotective effects and improves cognitive impairment in in vitro and in vivo Alzheimer's disease models. Mol Pharmacol. 2006;69(1):76–84.
14. Guzman-Esquivel J, Mendoza-Hernandez MA, Tiburcio-Jimenez D, Avila-Zamora ON, Delgado-Enciso J, De-Leon-Zaragoza L, et al. Decreased biochemical progression in patients with castration-resistant prostate cancer using a novel mefenamic acid anti-inflammatory therapy: A randomized controlled trial. Oncol Lett. 2020;19(6):4151–60.
15. Ayoub R, Jarrar Q, Ali D, Moshawih S, Jarrar Y, Hakim M, et al. Synthesis of Novel Esters of Mefenamic Acid with Pronounced Anti-nociceptive Effects and a Proposed Activity on GABA, Opioid and Glutamate Receptors. Eur J Pharm Sci. 2021;105865.
16. Jilani JA, Pillai GK, Salem MS, Najib NM. Evaluation of hydroxyethyl esters of mefenamic acid and diclofenac as prodrugs. Drug Dev Ind Pharm. 1997;23(3):319–23.
17. Shah K, Gupta JK, Chauhan NS, Upmanyu N, Shrivastava SK, Mishra P. Prodrugs of NSAIDs: a review. Open Med Chem J. 2017;11:146.
18. Ullah N, Huang Z, Sanaee F, Rodriguez-Dimitrescu A, Aldawsari F, Jamali F, et al. NSAIDs do not require the presence of a carboxylic acid to exert their anti-inflammatory effect–why do we keep using it? J Enzyme Inhib Med Chem. 2016;31(6):1018–28.
19. Couto M, Cates C. Laboratory guidelines for animal care. In: Vertebrate Embryogenesis. Springer; 2019. p. 407–30.
20. Smith ER, Hadidian Z, Mason MM. The single–and repeated–dose toxicity of dimethyl sulfoxide. Ann N Y Acad Sci. 1967;141(1):96–109.
21. Bose S, Mandal SK, Das P, Nandy S, Das A, Dutta D, et al. Comparative Evaluation of Anti-inflammatory, Antipyretic and Analgesic Properties of Ixora coccinea and Mussaenda frondosa (Rubiaceae) Leaves. Jordan J Pharm Sci. 2020;13(3).
22. Sultana T, Hossain ML, Chowdhury SA. Evaluation of Analgesic and Neuropharmacological Activity of the Bark of Morus alba L.(Family: Moraceae). Jordan J Pharm Sci. 2020;13(1).
23. Rahman M, Majumder S, Akter F, Islam F, Shahriar M, Alam J. Pre-clinical investigation of analgesic, anti-diarrheal and CNS depressant effect of Pterocarpus indicus in Swiss albino mice. Jordan J Pharm Sci. 2021;14(1).
24. Hunskaar S, Fasmer OB, Hole K. Formalin test in mice, a useful technique for evaluating mild analgesics. J Neurosci Methods. 1985;14(1):69–76.
25. Zawilska JB, Wojcieszak J, Olejniczak AB. Prodrugs: a challenge for the drug development. Pharmacol reports. 2013;65(1):1–14.
26. Vardanyan RS, Hruby VJ. Fentanyl-related compounds and derivatives: current status and future prospects for pharmaceutical applications. Future Med Chem [Internet]. 2014 Mar;6(4):385–412. Available from: https://pubmed.ncbi.nlm.nih.gov/24635521
27. Qandil AM. Prodrugs of nonsteroidal anti-inflammatory drugs (NSAIDs), more than meets the eye: a critical review. Int J Mol Sci [Internet]. 2012 Dec 17; 13(12): 17244–74. Available from: https://pubmed.ncbi.nlm.nih.gov/23247285
28. Meurs A, Clinckers R, Ebinger G, Michotte Y, Smolders I. Seizure activity and changes in hippocampal

extracellular glutamate, GABA, dopamine and serotonin. Epilepsy Res. 2008;78(1):50–9.
29. Balsara JJ, Jadhav JH, Chandorkar AG. Effect of intraperitoneally administered GABA on haloperidol-induced catalepsy in the rat. Psychopharmacology (Berl). 1980;68(1):105–7.
30. Ossowska K, Wędzony K, Wolfarth S. The role of the GABA mechanisms of the globus pallidus in mediating catalepsy, stereotypy and locomotor activity. Pharmacol Biochem Behav. 1984;21(6):825–31.
31. Worms P, Lloyd KG. Influence of GABA-agonists and antagonists on neuroleptic-induced catalepsy in rats. Life Sci. 1978;23(5):475–7.
32. Navarro JF, Pedraza C, Dávila G, López MM. Effect of gammahydroxybutyric acid administration on catalepsy behaviour in female mice. Psicothema. 2000;12(1):113–5.
33. Halliwell RF, Thomas P, Patten D, James CH, Martinez-Torres A, Miledi R, et al. Subunit-selective modulation of GABAA receptors by the non-steroidal anti-inflammatory agent, mefenamic acid. Eur J Neurosci. 1999;11(8):2897–905.
34. Rossokhin A. The general anesthetic etomidate and fenamate mefenamic acid oppositely affect GABA AR and GlyR: a structural explanation. Eur Biophys J. 2020;49(7):591–607.
35. Levy L. Carrageenan paw edema in the mouse. Life Sci. 1969;8(11):601–6.
36. Morris CJ. Carrageenan-induced paw edema in the rat and mouse. Inflamm Protoc. 2003;115–21.
37. Posadas I, Bucci M, Roviezzo F, Rossi A, Parente L, Sautebin L, et al. Carrageenan-induced mouse paw oedema is biphasic, age-weight dependent and displays differential nitric oxide cyclooxygenase-2 expression. Br J Pharmacol. 2004;142(2):331–8.
38. Aydede M. Does the IASP definition of pain need updating? Pain reports. 2019;4(5).
39. Kidd BL, Urban LA. Mechanisms of inflammatory pain. Br J Anaesth. 2001;87(1):3–11.
40. Berry PH, Dahl JL. The new JCAHO pain standards: implications for pain management nurses. Pain Manag Nurs. 2000;1(1):3–12.
41. Inoue S, Taguchi T, Yamashita T, Nakamura M, Ushida T. The prevalence and impact of chronic neuropathic pain on daily and social life: a nationwide study in a Japanese population. Eur J Pain. 2017;21(4):727–37.
42. Muir WW. Physiology and pathophysiology of pain. In: American Association of Bovine Practitioners Proceedings of the Annual Conference. 2003. p. 33–5.
43. O'Neil CK, Hanlon JT, Marcum ZA. Adverse effects of analgesics commonly used by older adults with osteoarthritis: focus on non-opioid and opioid analgesics. Am J Geriatr Pharmacother. 2012;10(6):331–42.
44. Duthie DJR, Nimmo WS. Adverse effects of opioid analgesic drugs. BJA Br J Anaesth. 1987;59(1):61–77.
45. Collier HOJ, Dinneen LC, Johnson CA, Schneider C. The abdominal constriction response and its suppression by analgesic drugs in the mouse. Br J Pharmacol Chemother. 1968;32(2):295–310.
46. Gawade S. Acetic acid induced painful endogenous infliction in writhing test on mice. J Pharmacol Pharmacother. 2012;3(4):348.
47. Blumberg H, Wolf PS, Dayton HB. Use of writhing test for evaluating analgesic activity of narcotic antagonists. Proc Soc Exp Biol Med. 1965;118(3):763–6.
48. Shibata M, Ohkubo T, Takahashi H, Inoki R. Modified formalin test: characteristic biphasic pain response. Pain. 1989;38(3):347–52.
49. Hunskaar S, Hole K. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain [Internet]. 1987;30(1):103–14. Available from: https://www.sciencedirect.com/science/article/pii/0304395987900881

السمية والفعالية العلاجية لكل من حمض الميفيناميك وهيدروكسي إيثيل إيستر حمض الميفيناميك في الفئران: دراسة مقارنة داخل الجسم الحي

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ملخص

الخلفية العلمية: يتميز هيدروكسي إيثيل إستر حمض الميفيناميك، وهو أحد المشتقات غير التجارية من حمض الميفيناميك، بخصائص مقاومة ضد التحلل الإنزيمي في المحاليل المنظمة المختلفة وكذلك في بلازما الدم. وبالرغم من ذلك، لا تتوفر دراسات توضح التأثيرات البيولوجية لهذا المركب داخل الجسم الحي. يقدم هذا البحث دراسة حول التأثيرات العلاجية لهذا المركب وسميته داخل جسم الكائن الحي مقارنة مع حمض الميفينامك الشبيه له في التركيب الكيميائي.

منهجية البحث: تم تقييم السمية الحادة لمركب هيدروكسي إيثيل إستر حمض الميفيناميك ومقارنتها مع سمية حمض الميفينامك من خلال حقن جرعات مكافئة في مجموعات من فئران التجارب ورصد القياسات التالية: نسبة حدوث الإغماء التخشبي، درجة (شدة) نوبت الصرع، نسبة حدوث النوبة التوترية الرمعية، نسبة الوفيات، درجة (مقياس) التجهم والنشاط الحركي. إضافة إلى ذلك، تم تقيم التأثير المضاد للالتهابات لهذا المركب من خلال فحص وذمة القدم التي يسببها الكارجينان وتقييم التأثير المضاد للألم باستخدام فحص التلوي الذي يسببه حمض الخليك في الفئران.

النتائج: أظهرت الفئران المعالجة بمركب هيدروكسي إيثيل إستر حمض الميفيناميك أن نسبة حدوث كل من الإغماء التخشبي والنوبة التوترية الرمعية والوفيات بالإضافة إلى درجة (مقياس) الصرع والتجهم كانت أقل منها في الفئران التي عولجت بجرعات مكافئة من حمض الميفينامك. كما أظهرت النتائج أن العلاج بمركب هيدروكسي إيثيل إستر حمض الميفيناميك سبب نشاط مضاد للالتهابات مكافئ لنشاط حمض الميفيناميك في فحص وذمة القدم التي يسببها الكارجينان. كذلك أظهر العلاج بمركب هيدروكسي إيثيل إستر حمض الميفيناميك نشاط مضاد للألم فاق نشاط حمض الميفيناميك في تجربة التلوي الذي يسببه حمض الخليك في الفئران.

الاستنتاج: تدل نتائج البحث على مقدرة هيدروكسي ايثل استر حمض الميفينامك على احداث نشاط علاجي اعلى من نشاط حمض الميفيناميك وتأثيرات جانبية أقل على الوظائف العصبية والعضلية.

الكلمات الدالة: حمض الميفيناميك، هيدروكسي ايثيل ايستر حمض الميفيناميك، نوبة الصرع، الاغماء التخشبي، مضادات الألم.

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