Development, Characterization, and Pharmacological Investigation of Sesamol and Thymol Conjugates of Mefenamic Acid

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ABSTRACT

BACKGROUND

Prodrug technology was extensively employed in the drug discovery processes and many approved drugs in the pharmaceutical industry were developed by the prodrug based synthetic approach. The current research work investigates the effect of the prodrug approach on the mefenamic acid by synthesizing the ester conjugates with natural antioxidants such as sesamol and thymol.

METHODS

Synthesis of two ester prod rugs, mefenamic acid-sesamol conjugate and mefenamic acid-thymol conjugate by coupling method using DCC / DMAP, subjected to physical-chemical characterization, spectral characterization (IR, ^{1}H NMR, ^{13}C NMR and Mass spectra), in-silico studies, in-vivo biodistribution studies and pharmacological evaluation such as anti-inflammatory, ulcerogenecity, activity in the brain as well as histopathological evaluation.

RESULTS

The ester prodrugs of mefenamic acid which upon administration would release the parent drug as a result of enzymatic or non-enzymatic hydrolysis in the desired areas with enhanced anti-inflammatory activity and reduction in the gastro intestinal toxicity. In-silico studies showed the docking score of mefenamic acid on the beta-secretase enzyme is - 7.834 and the bio-distribution study showed the enhanced distribution of the mefenamic acid in the brain. Pharmacological study and histopathology studies using the brain tissues showed the protective effect of mefenamic acid in the brain.

CONCLUSIONS

Antioxidant conjugates of mefenamic acid showed sustained release of the mefenamic acid and enhanced anti-inflammatory activity with reduction in the gastric toxicity. The present investigation also revealed that the enhanced transport profile across blood brain barrier and considerable protective effect in the brain against neurodegenerative conditions.

KEY WORDS

Pro Drug, Anti Inflammatory, Ulcerogenecity, Antioxidants, Neurodegeneration

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BACKGROUND

Mefenamic Acid, (MA) [2- (2, 3-dimethylphenyl)] amino benzoic acid) is a Non-Steroidal Anti-Inflammatory Drug (NSAIDs) having pharmacological activities like analgesic activity, anti-inflammatory activity, anti-pyretic activity and used for treating the muscular aches, menstrual cramps, head ache and dental pain.[1] The structure activity relationship of the mefenamic acid proved the null effect of free carboxylic acid functional group in the anti-inflammatory activity but several side effect is there for producing the gastric toxicity such as ulceration.[2] So the prodrug based synthetic approach masked the free carboxyl functional group by producing the ester conjugates of MA, MF-S and MF-T. The literature survey also showed the significant role of NSAIDs in the brain against neurodegenerative conditions but that is limited due to the hydrophilic nature. So, the prodrug based synthetic approach converted them into lipophilic and that is having the ability to cross BBB (Blood Brain Barrier).[3]

The conjugates used for synthesizing the ester prodrugs of MA were the natural antioxidants sesamol and thymol. The use of natural antioxidants in the diet can reduce the risk of various serious diseases conditions by scavenging the free radicals from the tissues and prevent oxidation. Most of the natural antioxidants are polyphenols, carotenoids and vitamins and in prod rug synthetic scheme, the use of this has conjugates can diminish the risk factor after hydrolysis of the prod rug at the site of action. Sesamol and thymol are phenolic natural antioxidants having wide variety of pharmacological activities. Literature survey showed that sesamol is having wide range of pharmacological activities such as anti-oxidant, anti-cancer, neuro-protective activity, cardio-protective, anti-inflammatory, anti-depressant, anti-amnesia, anti-ulcer, anti-anxiolytic etc.[4] Thymol also having several therapeutic activities such as anti-inflammatory, anti-oxidant, anti-hyperlipidaemic etc.[5] So the utilization of natural antioxidants as conjugates may produce synergistic pharmacological activity and the improved transport properties with the reduction of side effects.

Prodrug technology was extensively used in the drug discovery processes and many approved drugs in the pharmaceutical industry were developed by the prodrug-based concept. The usefulness and importance of this prod rug technology can be understood from the medicines used in the market such as angiotensin converting enzyme inhibitors, sulphasalazine for the management of ulcerative colitis, estradiol, dopamine etc. But the survey also indicated that limited clinical trials were conducted during past some years from the drugs developed from prodrug-based technology. But the prodrugs can be successfully overcoming the barriers of drug targeting and drug delivery to elicit the desired pharmacological activity. Prodrug are the inactive form of the active ingredient and that release the active drug by enzymatic or chemical conversion.[6] Prodrug technology is effectively utilized for synthesizing the antioxidant conjugates of MA that can overcome the side effects of NSAIDs and produced the enhanced therapeutic profile. The use of NSAIDs showed many side effects such as gastro-intestinal bleeding, cardio-vascular side effects, and drug induced nephro-toxicity etc.[7] among that, many side effects can be reduced by the prodrug technology through the synthesis of ester and amide prodrug. Ester prodrug having many advantages over other linkages such as susceptible to hydrolysis and yield good drug concentration after absorption and showed resistance to hydrolysis at absorption phase. This research work subjected to the modification of the mefenamic acid with the natural antioxidants, sesamol and thymol, synthesized two ester prodrug that is having enhanced therapeutic profile, transport profile and reduction in the toxicity profile.

METHODS

Chemicals and Instruments

The drug MA was obtained from TCI (Tokyo Chemical Industry) Chemicals (India) Pvt. Ltd., Chennai, Tamilnadu. The antioxidant 4-methyl umbelliferone was obtained from Sigma Aldrich Chemicals Pvt. Ltd. Mumbai. The FTIR spectra of the compounds were recorded on IR spectrometer (Bruker, software: Opus), at Al Shifa College of Pharmacy, Kerala. The elemental analysis by using elemental analyser (Thermo Finnigan, Italy, FLASH EA 1112 series) was done in Sophisticated Analytical Instrumentation Facility (SAIF), Lucknow.[1] H NMR (cryo-magnet spectrometer, Bruker), CNMR spectra (cryo-magnet spectrometer, Bruker) and MASS spectra (Micromass Q-Tof Micro) were performed in SAIF Panjab University, Chandigarh. The melting points of the prodrug was recorded using melting point apparatus (Sigma scientific products, Tamilnadu), Al Shifa College of pharmacy, Kerala. The absorbance was measured in the UV spectrophotometer (Shimadzu, Japan). Determination of physicochemical properties and the pharmacological evaluations were carried out in Department of Pharmaceutical Chemistry and Department of Pharmacology, Al Shifa College of Pharmacy. The histo-pathological studies were carried out in department of Pathology, KIMS and Al Shifa hospital, Kerala.

Synthesis of MA-Antioxidant Prodrug

This research work developed three ester prodrugs of NSAID, mefenamic acid by conjugating with two antioxidants sesamol and thymol thus mefenamic acid-sesamol prodrug [MF-S], and mefenamic acid–thymol prodrug [MF-T]. The method used for the synthesis of ester prodrugs was DCC / DMAP coupling method. To a stirred solution of 10 mmol of carboxylic acid in 15 ml of anhydrous dichloromethane (DCM) 110 mg DMAP and 10 mmol of sesamol was added. Then 10 mmol of DCC was added to the reaction mixture at 0 - 8^0 C, which is then stirred for 5 minutes at 0 - 8^0 C and for 3 hr. at 20 - 25^0 C. After completing the reaction, the precipitated urea was filtered off and the filtrate was evaporated. The residue was taken in 10 ml DCM and washed with saturated sodium bicarbonate solution and then dried over magnesium sulphate. The solvent is removed by evaporation and the ester isolated by recrystallization. Before the recrystallization the product was washed with alcohol to remove excess of sesamol. If the product after synthesis was sticky, it was washed with petroleum ether two or three times. The same procedure was used for the synthesis of MF-T and that is shown in the scheme of the synthesis.[8,9]

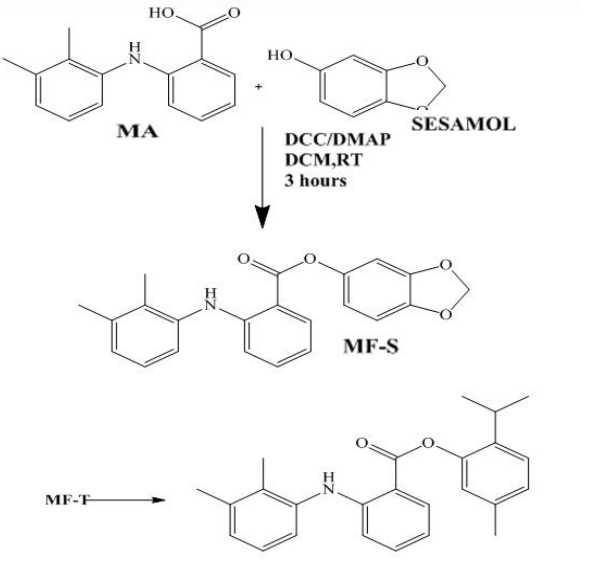

Figure 1. Scheme for the Synthesis of MF-S and MF-T

Physical-Chemical Characterization of MA, MF-S and MF-T

The physical as well as chemical properties of the synthesized prodrugs was done by different methods such as solubility, thin layer chromatography, partition coefficient determination, melting point determination, elemental analysis and spectral characterization and the produced data was compared with that of the parent drug, mefenamic acid. Solubility is an important parameter in the case of drugs that can be used for the formulation. In this study the drug and synthesized compounds were subjected to solubility analysis in different solvents such as 0.1 N NaOH, 0.1 N HCl, ethanol, methanol, ether, ethyl acetate, chloroform, acetone, DMF and water.[10] Thin layer chromatography was done to check the progression of the reaction and also confirm the purity of the synthesized compounds that was done on the pre coated silica G plates. The visualization was done by UV chamber. The solvent system used here was ethyl acetate: hexane 1:2.[11] The determination of melting point of the synthesized compounds was compared with that of the reactants to confirm the formation of the product and also assure the purity of the synthesized compounds.[12] The determination of partition coefficient aim is to attain the knowledge of the lipophilic profile of the synthesized compounds and that was done by shake flask method in which n-octanol saturated with phosphate buffer (pH 7.4). The concentration of the drug and each prodrug was monitored by measuring the absence using a UV-VISIBLE spectrometer.[13] The elemental data analysis was done to find the percentage of C, H, O and N in the prodrugs. The structure of the synthesized compounds was confirmed by different spectral analysis such as IR, ^{1}H NMR, ^{13}C NMR spectra and Mass spectra. The spectral data of the prodrugs were compared with that of the standard MA affirming the formation of the compounds by the scheme 1.

Molecular Modelling and ADME Studies

The docking studies were conducted to know about the interaction of the NSAID, MA in beta-secretase enzyme which is a cleavage enzyme of amyloid precursor protein. The docking score of the drug in the beta-secretase enzyme was found out by Ligprep, Glide, Prime, and Virtual Screening Workflow. Optimization of ADME is very much significant in the drug development process. QikProp is an in-silico method used for ADME optimization analysis of active lead molecules. All the synthesized molecules were subjected to pharmacokinetic parameters such as absorption, distribution, metabolism and excretion by use of Qikprop (Schrodinger, USA, 2015).[14]

Bio-Distribution

The bio-distribution of the synthesized prodrugs in the brain was done by in-vivo method and the parameter used to assess the brain distribution of the conjugates by Brain Targeting Efficiency (BTE = concentration of drug in the brain / concentration of the drug in the plasma) and that was done as per the procedure explained in Xuan Zhang et al., 2012.[15]

Pharmacological Evaluation

The ester prodrugs of MA were subjected to anti-inflammatory activity, anti-ulcerogenecity and analysis of biochemical parameters. The Wistar albino rat was used for the study and all the animal experiments were conducted after obtaining the institutional ethical committee approval (Reg.No. - 1195/PO/Re/S/08/CPCEA), Al Shifa College of pharmacy, Kerala.

Anti-Inflammatory Activity

The screening of anti-inflammatory activity was done by using carrageenan induced paw oedema method. In this screening method, inflammation was induced by 0.1 ml, 1 % w / v of carrageenan was used as inducing agent. Normal saline treated group act as control group and initially the volume of paw was measured using Vernier calliper. MA (50 mg / Kg) was administered for the standard group and corresponding doses of prodrugs were administered to the respective groups. After 30 minutes of administration, the carrageenan solution in normal saline was administered to each group. The volume of swelling was measured at 0, 1, 2, 4 and 6 hours and the mean increase in the volume of test compared with that of the standard.[15] Percentage inhibition was calculated by (1 - a / b) 100 where 'a' is mean increase in the paw thickness of treated groups and 'b' is the mean increase in the paw thickness of the control group.[16]

Anti-Ulcerogenic Study

Gastrointestinal toxicity expressed as lesions produced by the drugs and prodrugs and the mucosal damage was examined by using of an electron microscope. The severity of the gastric toxicity was measured by the parameter mean ulcer index and that was calculated by the following way. Grade 1: 1 mm erosions, grade 2: 1 – 2 mm erosions and grade 3: more than 2 mm erosions. The UI was calculated as UI = [1 × (number of lesions of grade 1) + 2 × (number of lesions of grade 2) + 3 × (number of lesions of grade 3) / 10].[17]

Activity in Brain

The distributed NSAIDs showed the activity in the brain can be evaluated by using behavioural test, antioxidant test and histopathology of the brain cortex valuation. The model used for pharmacological screening was aluminium chloride induced neurotoxicity model.[18] The animals were divided into five groups and each contain six animals. The Group I received normal saline which acts as control, Group II received aluminium chloride (50 mg / Kg) that acts as a negative control, Group III, IV and V received MA, MF-S and MF-T respectively. This chronic neurotoxicity model was conducted and evaluated for 90 days.

Behavioural Tests

Open Field Tests

Rats were placed in the open field apparatus and after placing the animals, they were allowed to move without any disturbance for 5 minutes and number of head dips, line crossing and rearing were counted.[19]

Marble Burying Assay

The marble burying test is a simple behavioural test conducted in rodents, especially rats and mice are exposed to glass marbles placed on thick bedding materials. Thirty clean glass marbles were arranged evenly on the bedding. After 30 minutes exposure to the marbles, mice were removed, and unburied marbles were counted. A marble was considered buried if its two-third size was covered with saw dust and the total number of marbles buried was considered as an index of locomotion.[20]

Water Maze Test

The rats were placed in the apparatus and escape latency was monitored. The apparatus consists of a large circular pool including a wooden material below. The experiment was conducted by the procedure explained by Nunez J et al.[21]

Antioxidant Parameters

For the in vivo test of antioxidant parameters, after behavioural study the mice were sacrificed, and brain tissue homogenate was prepared with normal saline and centrifuged. The supernatant was used for the tests. The superoxide dismutase and catalase were tested as per the procedure explained in the Khan R A et al., 2012 and Portia et al., 2018 respectively. Statistical significance of all studies was done by ANOVA and the values were expressed as mean ± SD[22,23,24]

Histopathology of Brain Cortex

Histopathology of the brain cortex of the different treated groups were examined by haematoxylin-eosin stain and monitored under electron microscope.[25]

Statistical Analysis

Results were communicated as mean ± SD of 6 rats in every group. The statistical significance between the groups was analyzed by utilizing One Way Analysis of Variance (ANOVA), followed by Dunnett's multiple correlation test. Significance level was fixed at 0.05.

RESULTS

Physical and Chemical Characterization

The two ester prodrugs MF-S and MF-T were successfully synthesized, and characterization was done, and the results were given in table 1. The two ester prodrugs, MF-S and MF-T were pale yellowish in colour and showed remarkable solubility in organic solvents than aqueous solvents. The significant difference in the melting point of the products from the reactants and single spot obtained in the thin layer chromatographic studies revealed the preliminary knowledge about the synthesis and purity of the MF-S and MF-T. The log P value of the synthesized compounds and the MA was done by partition coefficient study and indicated the enhanced lipophilicity of the synthesized ester prodrugs. The spectral studies confirmed the structure of the synthesized MF-S and MF-T.

Prodrug	Molecular weight	Colour	Melting Point	Log P	% yield	Rf Value	Elemental Analytical Data		
								Calculated %	Found %
MF-S [$C_{22}H_{19}NO_4$]	361	Pale yellowish	104-105^{0}C	1.39	72	0.75	C	73.12	73.23
							H	5.30	5.38
							F	3.88	3.34
							O	17.71	17.74
MF-T [$C_{25}H_{27}NO_2$]	373	Yellowish	88-90	1.23	73	0.64	C	80.40	80.35
							H	7.29	7.32
							N	3.75	3.81
							O	8.57	8.52

Table 1. Physical and Chemical Characterization of MF-S and MF-T

MF-S (benzo [d] [1,3] – dioxol -5-yl-2- ((2,3-dimethyl phenyl) amino) benzoate): FTIR (cm^{-1}, KBr): 3317 (-NH), 2924 (aromatic C–H), 2853 (Aliphatic C-H) and 1684 (C O ester); ^{1}HNMR ($CDCl_3$,):2.31 (S, 3H), 2.15 (S, 3H), 5.99 (S, 2H in the dioxole ring), 6.80 (d, J = 8.5, benzene ring 1H), 6.73 (t, J = 6.8, 1H), 6.82 (d, J = 8.35, 1H), 8.15 (d, J = 6.7, 1H), 7.30 (t, J = 7.1, 1H), 7.10 (t, J = 7.1,1H), 7.15 (d, J = 7.75, 1H), 9.16 (s, 1H), 6.65 (d, J = 8.35, 1H), 7.01 (d, J = 7.35, 1H), 6.70 (s, 1H); Mass (m / z) :362; ^{13}CNMR:14.03, 20.64, 101.77, 104.19, 108.02, 109.58, 113.82, 114.39, 116.21, 123.22, 125.99, 127.05, 131.89, 132.57, 135, 138.31, 138.39, 145.07, 145.48, 148.19150.26, 167.72.

MF-T (2-isopropyl-5-methyl phenyl2- ((2,3-dimethyl phenyl) amino) benzoate): FTIR (cm^{-1}, KBr): 3324 (-NH) 3070 (aromatic C–H), 2921(Aliphatic C-H) and 1671 (C O ester); ^{1}HNMR ($CDCl_3$,):9.23 (S, 1H), 8.22 (d, J = 6.55, 1H), 7.32 (t, J = 7.2, 1H), 7.25 (t, J = 6.8, 1H), 7.19 (d, J = 7.8, 1H), 7.10 (d, J = 7.6, 1H), 7.08 (d, J = 8, 1H), 7.02 (d, J = 7.45, 1H), 6.95 (s, 1H), 6.83 (d, J = 8.55, 1H), 6.74 (t, J = 7.15, 1H), 3.10 (m, J = 6.9, 1H), 2.35 (s, 3H), 2.31 (s, 3H), 2.17 (s, 3H),1.24 (dd, J = 6.9, 6H); ^{13}CNMR:167.60, 150.21, 147.98, 138.48, 138.29, 137.53, 136.68, 134.87, 132.44, 131.86,127, 126.92, 126.55, 125.93, 123.08, 123.07, 116.26, 113.84, 10.9.88, 27.26, 23.16, 23.16, 20.92, 20.64, 14.01; Mass (m / z):374

Molecular Modelling and ADME Studies

The molecular modelling study revealed that the NSAID, MA is able to bind to the active site of the Beta-Secretase enzyme, with a docking score of - 10.349 and - 7.834 respectively. In-silico and molecular modelling studies showed that in addition to its anti-inflammatory activity it also inhibits the beta

secretase enzyme which produces the beta amyloid protein in the neuronal cells. So based on the results, if these molecules can directly target in to the brain may give potential therapeutic activity in the degenerated conditions.

Also the pharmacokinetic parameters were found out by Qikprop and the predicted MDCK permeability of blood brain barrier was find out and the range accepted was below 25 is poor, above 500 is great that also indicated the enhanced permeability of the prodrugs ie MA-193.17, MF-S-3138.16 and MF-T-4189.51. The pharmacokinetic parameters showed significant variation of the values between the drugs and the synthesized ester compounds, MF-S and MF-T.

Bio-Distribution and Brain Targeting Efficiency

The distribution of the drug and prodrugs can be evaluated by bio-distribution conducted in vivo method. The parameter used for evaluating the brain distribution of the drug and pro drugs is brain targeting efficiency. The $(C_{brain} / C_{plasma})_{MA}$ ratios of the 10 min after administration MA, MF-S and MF-T pro drugs were 0.046 ± 0.014, 0.491 ± 0.013 and 0.1366 ± 0.015 respectively.

Pharmacological Evaluation

Anti-Inflammatory Activity

MA and the two natural antioxidants are having anti-inflammatory activity and the prodrug technology revealed the enhanced anti-inflammatory activity provided by the two ester prodrugs MF-S and MF-T. The results of the comparative approach were shown in the table 2. From the six hours of observations, it was concluded that the two ester prodrugs showed about 70 percentage of activity compared with that of the parent drug thus provide synergistic activity and also the study proved the sustained release of the drug and conjugate.

Group	Prodrug	Dose (mg p.o.)	Anti-Inflammatory Activity (%)[b]					Mean Ulcer Index
			1 h	2 h	3 h	4 h	6 h	
I	Normal Control[a]	Normal saline	-	-	-	-	-	0
II	MA	8.0	49.0 ± 1.2	48.0 ± 1.1	45.8 ± 1.9	43.3 ± 1.2	42.5 ± 1.4	23.9± 1.2
III	MF-S	10.5	44.1 ± 1.3[c]	51.2 ± 1.1[c]	60.0 ± 1.2[c]	69.3 ± 1.3[c]	75.5 ± 1.4[c]	4.5 ± 1.5[b]
IV	MF-T	13.0	42.0 ± 1.5[c]	51.0 ± 1.7[c]	56.8 ± 1.8[c]	59.4 ± 1.1[c]	68.7 ± 1.4[c]	5.1± 1.41.1[b]

Table 2. Data of Anti-Inflammatory Activity

[b]Each value represented as mean ± SD of six observed data. [c]represents the comparison between the groups III, IV vs. group II. Also, [b]represents the comparison between the groups III, IV vs group II. [c]p, [b]p < 0.05 and significance found out by ANOVA followed by Dunnett's test.

Ulcerogenic Activity

The main risk factor of NSAIDs is the production of gastric ulcers and this study made comparison between the production of ulcers by the parent drug, MA and the antioxidant conjugated MA derivatives through the parameter mean ulcer index. The ulcer production was visually monitored. The MA has the mean ulcer index 23.9 and MF-S and MF-T have 4.5, 5.1 respectively. The comparative study revealed the prodrug-based approach successfully reduces the side effect, gastric ulceration, produced by the MA.

Activity in Brain

Pharmacological investigation was done for monitoring the behavioural parameters, biochemical evaluation i.e. antioxidant parameters and histopathology of brain to confirm the activities of the synthesized pro drugs. The results were in the Table 3.

Group	Open Field Test			Marble Burying Test	Water Maze Test (Time in Seconds)	Antioxidant Activity [U / mg Protein]	
	Head-Dips	Rear-ing	Line Crossing			SOD	Catalase
I	9.0 ± 0.058	22.0 ± 0.068	36.0 ± 0.039	28.0 ± 0.043	55.18 ± 0.089	7.66 ± 0.042	2.31 ± 0.089
II	2.0 ± 0.046[a]	8.0 ± 0.063[a]	10.0 ± 0.041[a]	7.0 ± 0.078[f]	120.76 ± 0.076[m]	1.54 ± 0.046[a]	0.43 ± 0.059[a]
III	3.0 ± 0.047[b]	9.0 ± 0.068[b]	13.0 ± 0.044[b]	10.0 ± 0.087[k]	111.93 ± 0.072[n]	1.99 ± 0.037[b]	0.49 ± 0.061[b]
IV	7.0 ± 0.044[c]	17.0 ± 0.050[c]	31.0 ± 0.057[c]	22.0 ± .069[g]	64.58 ± 0.064[p]	5.69 ± 0.053[c]	1.38 ± 0.077[c]
V	4.0 ± 0.057[c]	10.0 ± 0.065[c]	19.0 ± 0.063[c]	14.0 ± 0.088[g]	88.68 ± 0.049[s]	2.93 ± 0.022[d]	0.98 ± 0.065[d]

Table 3. Results of the Behavioural Tests and Antioxidant Activity

Marble Burying Test

The values were expressed as the mean ± SEM of six animals. [f]P < 0.001 represents the significance of group II with Group I. [g]P < 0.05 represents the significance on Group V with IV. [k]P < 0.01 represents statistical significance of group III vs II.

Water Maze Test

Each value was expressed as mean ± SEM of six animals. [m]p < 0.01 for comparison of group I with the Group II.[n] p < 0.001 for Group III with Group II. [p]P < 0.05 represents the significance on Group IV with III. [s]P < 0.01 represents statistical significance of group V vs. III.

Antioxidant Activity

Each value is expressed as the mean ± SEM of six animals. [a]p < 0.01 for comparison of group I with the Group II. [b]p < 0.001 for Group III with Group II. [c]P < 0.05 represents the significance on Group IV with III. [d]P < 0.01 represents statistical significance of group V vs. III.

Behavioural parameters were monitored by using Open field test, Marble burying test and water maze test. In open field studies, the number of head dipping, rearing and line crossing were considered for the assessment of locomotor and cognitive function. The oral administration of aluminium chloride significantly (P < 0.001) induced the neurotoxicity when compared to the normal saline treated animals (control group). The MA treated animals showed no significant improvement in the activity profile that indicated the very limited distribution of the drug in the brain. But MF-S and MF-T prodrugs showed improvement in habituation memory compared with the parent drug that was shown in the table 3.

Behavioural data obtained from the marble burying test is a parameter to test the locomotion. The result showed that aluminium chloride induced group had less locomotion

compared with the control. But the synthesized ester pro drugs treated groups showed better activity than the parent drug treated group and that was shown in the table 3.

The water maze test evaluated the spatial learning and memory and the results were tabulated in table 3 and graphically represented in table 3. The results showed that the negative control group has increased escape latency compared to the control group ($p < 0.01$). The time taken to escape is decreased in the case of prodrug treated groups compared with that of MA treated groups. The synthesized ester pro drugs significant decrease in the escape latency indicated the protective effect of prodrugs in brain.

The evaluation of the antioxidant parameters showed that the enhanced activity of the natural antioxidant conjugated ester prodrugs and that provided the protective effect against the degeneration in the nerve cells. The data was given in table 3. Histopathology of brain cortex also revealed the protective nature of MA against the aluminium chloride neurode generation by conjugating with phenolic antioxidants.

Open Field Test

The values are expressed as the mean ± SEM of six animals. Superscripts showed the statistical significance by ANOVA followed by Dunnett's test. $^{a}P < 0.001$ represents the significance of group II with Group I. $^{b}P < 0.05$ represents the significance on Group III with II. $^{c}P < 0.01$ represents statistical significance of group IV, V vs. III.

Histopathology

Histopathology of mice brain were taken from the normal saline group, negative control group, MA, MF-S and MF-T treated groups and the results were analyzed. In the prodrug treated group, all the two different ester prodrugs shown normal cells of cortex without any spongiform cells, indicated the protective effect of synthesized natural compound conjugating ester prodrugs.

DISCUSSION

The two natural phenolic antioxidant conjugated mefenamic acid prodrugs were synthesized and physical chemical characterization confirmed the structure and purity of the novel compounds, MF-S and MF-T.

The MF-S and MF-T produced enhanced anti-inflammatory activity and showed a sustained release pattern by using carrageenan induced paw oedema method. Many studies showed that the carrageenan inducing the paw oedema. The oedema induced by carrageenan produced acute and local inflammatory response.[26] The conjugates used in this study, sesamol and thymol, also produced anti-inflammatory activity and antioxidant processes.[27] Therefore overall, the synthesized products showed synergistic anti-inflammatory activity.

There are many report suggesting that the main side effect of NSAIDs is the production of gastric ulceration because of the free carboxyl functional group in the NSAIDs.[28] So the prodrug based synthetic approach on MA by using antioxidant showed significant reduction of gastric ulcer formation due to the modification of the free carboxylic acid group present in the NSAID and also by the protective nature of antioxidants.

MF-S and MF-S showed the significant protective effect against the neurotoxicity produced by the aluminium chloride. Literature survey suggested that the $AlCl_3$ alters the behavioural and neurotransmitter as well as learning and memory.[29] Aluminium gets deposited in brain regions, after exposure to long duration, specifically in hippocampus and cortex and leads to the degenerative diseases such as AD (Alzheimer's Disease) and PD (Parkinson's Disease).[30,31] There are several studies that revealed the aluminium toxicity in brain leads to cognitive dysfunction[32] and locomotor problems.[33] The effect in the brain can be tested by the behavioural tests such as open field test, Marble burying test[34] and Water maze test[35] in which the memory and motor activity was decreased. But it was observed that the considerable enhancement of the cognitive and motor activities in the prodrug treated groups. The reports showed that the antioxidant SOD (Superoxide dismutase)[36] and catalase[37] showed protective effect in brain against the degenerative conditions. In vivo antioxidant test of MF-S and MF-T done and the comparative study against the MA proved the increased SOD and catalase in the prodrug treated groups. The outcome of the present investigation proved the enhanced anti-inflammatory activity, reduction in gastric toxicity and protective activity in the central nervous system.

CONCLUSIONS

The ester prodrugs of MA were successfully synthesized, and the study proved the enhanced pharmacological activities of MA by prodrug approach with a reduction in side effects. Also, remarkable improvement in the transport properties across the BBB was seen. Pharmacological studies showed enhanced anti-inflammatory activity and significant decrease in the ulcerogenic index. This research work also attempted to reveal the role of NSAID in neurodegenerative conditions. This study showed remarkable brain distribution of the MF-S and MF-T in the brain with notable protective action against the neurodegenerative conditions. The prodrug based synthetic approach successfully attains the goal to improve the therapeutic and transport profile with a reduction in the toxicity profile of the NSAIDs.

Data sharing statement provided by the authors is available with the full text of this article at jemds.com.
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