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Studies on synthesis, stability, release and pharmacodynamic profile of a novel diacerein-thymol prodrug

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ABSTRACT

Involvement of oxidative stress, leading to chondrocyte senescence and cartilage ageing has been implicated in the pathogenesis of osteoarthritis (OA). New efforts to prevent the development and progression of OA include strategies and interventions aimed at reducing oxidative damage in articular cartilage using antioxidants as adjuncts to conservative therapy. Diacerein is an anthraquinone derivative with a marked disease modifying effect on OA owing to IL-1 β inhibition. In the present work an attempt was made at design and development of a co-drug of diacerein with antioxidant thymol. Structural elucidation was carried out by spectral analysis. When release kinetics of prodrug was studied in phosphate buffer (pH 7.4) and small intestinal homogenates of rats, 91% and 94% diacerein was available respectively at the end of 4.5 h. Chemical linkage of thymol with diacerein improved its lipophilicity and hence bioavailability. Screening of prodrug in Freud's adjuvant-induced arthritis and ulcerogenic potential by Rainsford's cold stress model exhibited significant reduction in paw volume, joint diameter and ulcer index with superior anti-inflammatory/anti-arthritic activities than the standards. Results of histopathology of tibio-tarsal joint indicated that animals treated with diacerein exhibited moderate synovitis while thymol and physical mixture-treated animals showed mild synovitis. Interestingly in prodrug-treated animals synovitis was not observed. The results of this study underline the promising potential of co-drug of diacerein and thymol in the management of OA.

Osteoarthritis (OA) is a degenerative type of arthritis in which the biomechanical properties of cartilage are altered due to its break down in synovial joints by local proteases. The involvement of mechanical and cytokine-mediated pathways has been emphasized in cartilage degeneration and pathogenesis of OA.[1] Signs and symptoms of inflammation, joint pain, swelling and stiffness causing significant functional impairment and disability are the highlights of OA. A common feature is infiltration of activated B cells and T lymphocytes and overexpression of proinflammatory mediators in early and late OA causing synovitis. This contributes to dysregulation of chondrocyte function that disturbs the balance between the catabolic and anabolic activities of the chondrocytes which is normally involved in remodeling the cartilage. There is a direct co-relationship between release of inflammatory mediators like prostaglandins, nitric oxide, IL-1β and tumor necrosis factor-α (TNF)-α in OA synovial fluid and joint tissue. The underlying mechanism is not well documented but involvement of abnormal mechanical, and oxidative stresses is indicated.[2]

Cartilage matrix component's degeneration and excessive production of different cytokines are the most prominent features of OA. Inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), microsomal prostaglandin E synthase-1 (mPGEs-1), and matrix metalloproteinases are the important pro-inflammatory mediators whose synthesis is stimulated by interleukin IL-1β, promoting the release of nitric oxide (NO) and prostaglandin E2 (PGE2).[3–6] Anabolic activities in chondrocytes are also retarded by IL-1 β causing decreased proteoglycan and collagen synthesis.[7–9]

The treatment is aimed at relieving pain and suffering, maintenance and restoration of function and ultimately prevention of disease progression.[10] The current treatment modalities for OA are either conservative or surgical. Conservative measures prominently involve pharmacological intervention in the form of nonsteroidal anti-inflammatory drugs (NSAIDs), intra-articular steroid injections or relatively new option of injection of hyaluronan which improves joint lubrication and can decrease pain. All these current treatments are basically symptomatic and do not retard destruction of articular cartilage. Therefore, there is an urgent need for therapeutic modalities to protect or induce regeneration of cartilage on a cellular level by restoring its structural integrity and function. Disease modifying anti-rheumatic drugs (DMARDS) are drugs that not only manage symptoms but also favorably affect joint structure changes over long-term treatment periods and thus

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slow or arrest disease progression, so also called structure modifying drugs.[11] Therefore, out of the critical need to develop alternative agents that prevent the destruction of cartilage and/or stimulate its proper repair, DMARDS came into picture.[12]

The pathophysiology of OA is complex with interplay of many converging mechanisms. The results of study performed by Surapaneni et al. suggest higher oxygen-free radical production and oxidative stress in OA.[13] Numerous reports have demonstrated that oxidative damage due to the over-production of nitric oxide (NO) and other reactive oxygen species (ROS) may be playing a significant role in the pathogenesis of OA and also in the formation of gastric mucosal lesions associated with NSAIDs therapy.[14–16]

The current, modern OA therapy has its own limitations in preventing joint destruction which is grossly focused on inhibiting formation of inflammatory mediators such as prostaglandins and leukotrienes with anti-inflammatory agents. With the understanding of the significant role of antioxidants in arthritis, they are gaining increasing importance as an adjunct therapy as they would help in protecting the biological tissues below a critical threshold of reactive oxygen species[17,18] by forming a mutually supportive defense team against ROS in OA. Impaired antioxidant defense and increased lipid peroxidation suggest that treatment with antioxidants at the initial stages of illness may prevent further oxidative injury and deterioration of associated musculoskeletal deficits in OA.[19] Oxidative stress leads to increased risk for OA but the precise mechanism remains unclear. The findings of Yudoh et al. (2005) clearly show that the presence of oxidative stress induces dysfunction of chondrocytes in OA cartilage, suggesting that oxidative stress, leading to chondrocyte senescence and cartilage ageing, might be responsible for the development of OA.[20] New efforts to prevent the development and progression of OA may include strategies and interventions aimed at reducing oxidative damage in articular cartilage.

In the last decade scientists have proven that some antioxidants have anti-inflammatory properties. In addition to scavenging free radicals, there are antioxidants that actually block inflammation. The antioxidant effect (the blocking of certain oxidizing proteins) lowers the activation of inflammatory signals.[21]

Diacerein is a very recently introduced, symptomatic slow acting disease modifying IL-1β inhibitor, known to possess antiarthritic and moderate anti-inflammatory, antipyretic and analgesic activities.[22] The mechanism of action of diacerein differs from those of NSAIDs or corticosteroids. Neither diacerein nor its active metabolite rhein, inhibit prostaglandin biosynthesis; indeed, cyclooxygenase/lipoxygenase pathways. This unique feature seems to be the reason for the excellent gastric safety profile of diacerein during OA treatment. But according to the theory of Cioli et al. diacerein might produce local irritant effect on the gastric mucosa due to its free carboxylic group.[23] It is reported that diacerein's disease modifying effect is more prominent while its anti-inflammatory effect is mild to moderate with a late onset.[22]

Dhaneshwar et al. (2009, 2012) have reported mutual prodrugs of diacerein with aminosugar as well as amino acids possessing lowered ulcerogenic tendency and more aqueous solubility. These prodrugs had quicker onset of action as compared to diacerein.[24–26] The present work was inspired by promising results of our earlier work. The role of increased levels of ROS and oxidative stress is well documented in the literature. Antioxidants have been suggested as a secondary therapy aimed at limiting tissue destruction.[18] Therefore, an antioxidant carrier; thymol was chosen as a carrier for its covalent linkage with –COOH group of diacerein so as to develop its mutual prodrug with reduced local irritant effect, improved absorption, prolonged release of drug and enhanced anti-inflammatory effect. It has been suggested that co-administration of antioxidants and NSAIDs in formulated dosage forms may possibly suppress the progression of OA and decrease the risk of NSAIDs-induced GI toxicity.[27] Thymol is reported to possess anti-oxidant/anti-inflammatory activity.[28–31] Thymol is listed by the US Food and Drug Administration (US-FDA) as a food additive on the 'generally recognized as safe' (GRAS) list therefore it would be nontoxic. Thymol is hydrophobic in nature[30] that might enhance lipophilicity of diacerein, in turn increasing its transcellular absorption.

In the present work, carboxylic group of diacerein was masked in a transient manner with antioxidant thymol. We expected that this conjugation would increase lipophilicity and absorption of diacerein, decrease the gastric irritant effect and enhance it is anti-inflammatory activity by reducing the oxidative stress in OA. Evaluation and comparison of the efficacy of this novel mutual prodrug with individual drugs and their physical mixture was also one of the objectives of this work.

Thymol was purchased from LOBA Chemie, Mumbai, India while diacerein was obtained as a gift sample from Glenmark Pharmaceutical Pvt. Ltd, Mumbai, India. Synthetic procedures were optimized on Radley's 6-station parallel synthesizer. Thin layer chromatography was performed on pre-coated silica gel plates-60 F264 (Merck) for purity check and monitoring of reactions. The IR spectrum of the synthesized compound was recorded on Jasco V-530 FTIR in anhydrous IR grade potassium bromide. The ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Avance II 400 NMR at Sophisticated Analytical Instrument Facility (SAIF), Panjab University, Chandigarh. Chemical shift values are reported in ppm downfield on δ scale. The absorbance maximum (λ_{max}) was determined on Jasco V-530 UV–Visible double-beam spectrophotometer. Partition coefficient was determined experimentally in *n*-octanol/phosphate buffer (pH 7.4) by flask shake method. In vitro release of diacerein from its prodrug and log*P* values were determined using Jasco V-530 UV–Visible double-beam spectrophotometer. Institutional Animal Ethical Committee's approved experimental protocols were followed for pharmacological screening of synthesized prodrug which was carried out with at the CPCSEA-approved animal facilities of Poona College of Pharmacy, Pune, India. Anti-arthritic activity was evaluated by Freund's adjuvant-induced arthritis using Complete Freund's adjuvant (F-5881, Sigma–Aldrich Corporation, USA). The body weight, joint diameter and paw volumes (both the paws) were measured using weighing balance, digital vernier caliper and UGO BASILE Plethysmometer 7140, Italy respectively. The photomicrographs were taken on inverted microscope attached with digital olympus model no: E-PL1 camera with 40× resolution.

Prodrug of diacerein with thymol (DTH) was synthesized by DCC coupling method[32] (Fig. 1) and then characterization of its physico-chemical properties was performed by spectral analysis.[33]

In vitro release profile of synthesized prodrug was investigated in aqueous buffers (HCl buffer pH 1.2 and phosphate buffer pH 7.4) and tissue homogenates of upper gastro-intestinal tract (GIT). As the difference between λ max of diacerein (258 nm) and DTH (230 nm) was substantial (28 nm), a new method was developed for simultaneous estimation of diacerein in presence of its prodrug DTH by employing UV spectrophotometer. The method was validated as per ICH guidelines and carried out in triplicate. The *K* values from the plots were calculated separately and average *K* and SD value was determined.

Release kinetics of the prodrug was studied in aqueous buffers of varied pH. DTH (10 mg) was introduced in 100 ml of HCl buffer[34] taken in different beakers kept in a constant temperature bath at 37 ± 1 °C. The solutions were occasionally stirred and 5 ml aliquot portions were withdrawn at various time intervals (0–180 min) each time replenishing with fresh 5 ml HCl buffer (pH 1.2). The aliquots were directly estimated on UV spectrophotometer at 232 nm for the amount of DTH remaining and at 259 nm for released diacerein. Same procedure as above was followed to study release

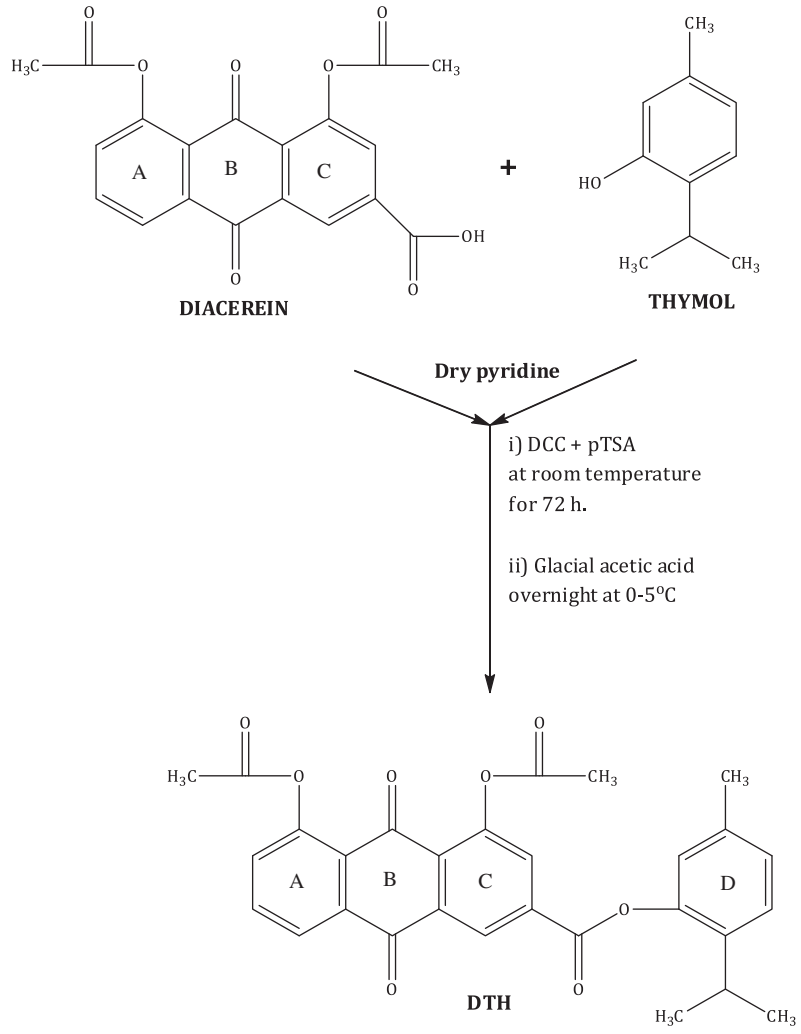

Figure 1. Scheme of synthesis.

kinetics in phosphate buffer pH 7.4 except that the HCl buffer was replaced by phosphate buffer pH 7.4[35] and release of diacerein was monitored over a period of 4.5 h at 230 nm for the amount of DTH remaining and at 258 nm for amount of diacerein released.

To study the release kinetics in upper GI homogenate, a male Wistar rat was anesthetized by ether and midline incision was made. The stomach and small intestine were separated out, homogenized with their contents and diluted to half concentration with isotonic hydrochloric acid buffer (pH 1.2) and phosphate buffer (pH 7.4) respectively. DTH was weighed (10 mg) and dissolved in HCl buffer (pH 1.2) and volume was made up to 10 ml (1000 μg/ml). 1 ml of this solution was added to 10 ml volumetric flask and volume was made up to 10 ml with HCl buffer (pH 1.2) (100 μg/ml). This was considered as the stock solution. To each eppendorf tube (1 ml); 0.8 ml of the stock solution of prodrug and 0.2 ml of stomach homogenate was added and kept in incubator at 37 °C. The Eppendorf tubes were taken out at appropriate time intervals, centrifuged at 10,000 rpm at 4 °C for 10 min, filtered through membrane filter and estimated at 232 nm and 259 nm taking HCl buffer as blank. Concentrations of DTH/diacerein at respective times were calculated from equations of their calibration curves in HCl buffer. For release studies in small intestinal homogenates, same procedure as above was employed for 4.5 h. Absorbance was observed at wavelength of 230 nm (for DTH) and 258 nm (for diacerein) taking phosphate buffer as blank. The results of overall kinetic studies are compiled in Table 1 and depicted graphically in Figure 2.

Anti-arthritic effect of DTH was investigated in Freund's adjuvant—induced arthritis as per protocols described by Vogel[35] and our earlier publication[24] Male Wistar rats (200–250 g) were divided into 6 groups viz healthy control, arthritic control which received FCA injection sub-plantar (day 1) + 0.25% of sodium CMC suspension p.o. (day 13–28), reference standard diacerein at the dose 5 mg/kg p.o. (day 13–28), thymol at the dose of 2.04/kg p.o. (day 13–28), physical mixture of diacerein and thymol at the dose

Table 1
Kinetics data for release of diacerein from its prodrug DTH at 37 °C

Medium	Order of kinetics	$t^{1/2}$ (min)	K (min^{-1})[a]	% Diacerein released over 4.5 h
HCl buffer (pH 1.2)	—	—	—	Stable
Stomach homogenates	—	—	—	Stable
Phosphate buffer (pH 7.4)	First	193	$3.59 \times 10^{-2} \pm 0.0001$	90.6
Small intestine homogenates	First	168	$4.12 \times 10^{-2} \pm 0.0001$	93.7

[a] Average of three readings.

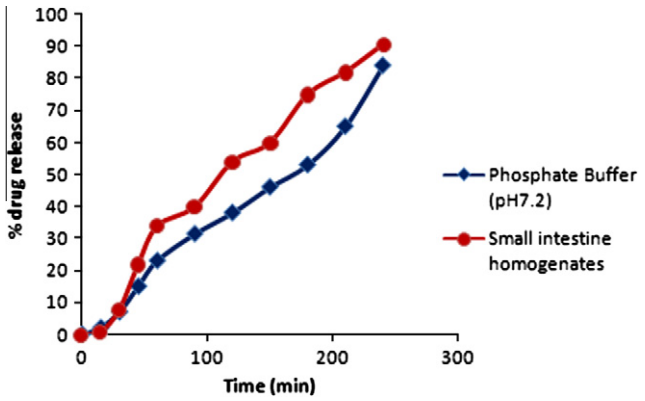

Figure 2. Release kinetics of DTH prodrug in phosphate buffer (pH 7.4) and small intestinal homogenates at 37 °C.

of 7.04 (5 + 2.04) mg/kg p.o. (day 13–28) and DTH at the dose of 6.8 mg/kg p.o. (day 13–28). The dose of prodrug was calculated on an equimolar basis to diacerein. Parameters studied under this model were change in body weight, joint diameter, paw volume and percent inhibition of edema which were measured on 0th, 3rd, 7th, 12th, 15th, 19th, 21st and 28th days using weighing balance, digital vernier caliper and UGO BASILE Plethysmometer 7140, Italy, respectively. Percent inhibition of edema was determined by the following formula:

$$\%\text{Inhibition of edema} = 1 - (V_t/V_c) \times 100$$

where V_t is the mean relative change in paw volume in test/standard group and V_c is the mean relative change in paw volume in arthritic control group. Average of six readings was calculated and all data were expressed as mean ± SEM. Statistical differences between the groups were analyzed using Graphpad Prism 5.0 software by two way ANOVA followed by BonFerroni's post test. The results of various parameters of anti-arthritic activity are summarized in Figures. 3–6.

All the animals were sacrificed on 28th day and samples of ankle joints were sent for histological studies. Ankle joints were separated from the hind paw, weighed and immersed in 10% buffered formalin for 24 h followed by decalcification in 5% formic acid, processed for paraffin embedding sectioned at 5 μ thickness and subsequently stained with haematoxylin and eosin for evaluation under light microscope. Sections were examined for the presence of hyperplasia of synovium, pannus formation and destruction of joint space. The photomicrographs were taken on inverted microscope attached with digital olympus model no: E-PL1 camera with 40× resolution. The photomicrographs of histopathological studies of rat ankle joint are depicted in Figure 7.

Orally administered diacerein induces moderate ulceration in the upper GIT due to its carboxylic group, temporary masking of which might lower its propensity for producing local ulcers. So to test our hypothesis, DTH was screened for its gastro-protective effect on GIT mucosa at ten times higher dose by Rainsford's cold stress method[36] as per protocols described by Dhaneshwar et al.[24] Suspensions of diacerein and test compounds in 0.25% CMC were administered orally. Male Wistar rats (200–250 g) were fasted overnight and divided into different groups ($n = 6$). Animals were treated with diacerein (50 mg/kg, p.o.) and equimolar dose of DTH (68 mg/kg) and physical mixture of diacerein + thymol (50 + 20.4 mg/kg). Control group was treated with an equal volume of 0.5% CMC solution. Images of stomach dissected from the sacrificed rats were captured using a Crystal Clear Display (CCD) Scanner (UMAX ASTRA 5600) at magnification of 2400 dpi. Images were analyzed using adobe photoshop and image software to calculate ulcer area and total area of each stomach. Ulcers were scored as per Cioli's scoring method.[24,37] Average of six readings was calculated and all data was expressed as mean SEM (Table 2). Statistical differences between the groups were calculated by One-Way ANOVA followed by Dunnett's post hoc test. Differences were considered at a p value of <0.01.

The log$P$ (n-octanol: phosphate buffer pH-7.4) of DTH was found to be higher (0.565) than diacerein (0.46) which would ensure better absorption and increased bioavailability in the body because of enhanced lipophilicity. The IR spectrum of the synthesized compound showed absorption band at 1780 cm^{-1} characteristic of C=O stretching of ester. Absence of phenolic OH stretching at 3219 cm^{-1} (thymol) and carboxylic OH stretching at 2553 cm^{-1} (diacerein) further confirmed the formation of an ester. The ^{1}H NMR spectrum of DTH showed chemical shifts for protons of isopropyl and methyl groups (thymol) as well as aromatic protons and protons of methyl ester (diacerein) which were characteristic of the anticipated structure of the prodrug. Chemical shifts for proton of –OH of –COOH of diacerein at 11 ppm and phenolic –OH of thymol at 5 ppm were totally absent in ^{1}H NMR of DTH that

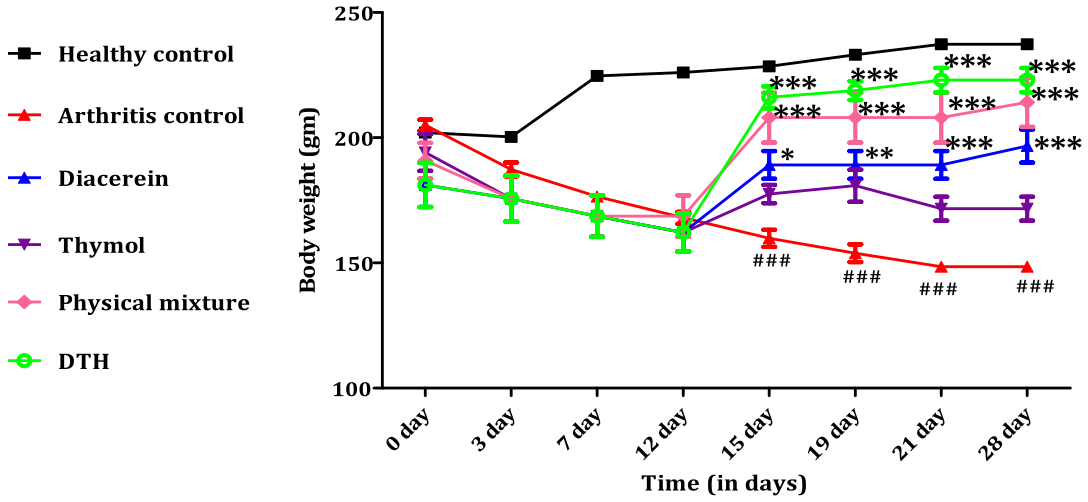

Figure 3. Effect of prodrug on improvement of body weight (g) of rats in FCA-induced arthritis. Values are expressed as mean ± SEM of six readings, $^{*}p$ <0.05, $^{**}p$ <0.01, $^{***}p$ <0.001 when compared to arthritic control and $^{\#\#\#}p$ <0.001 when compared to healthy control.

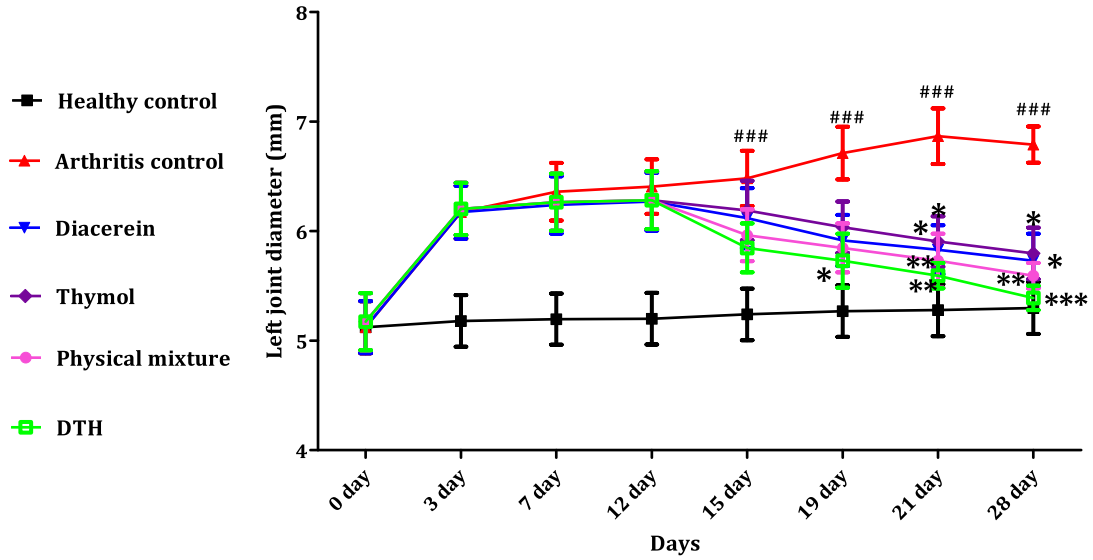

Figure 4. Effect of prodrug on reduction of left joint diameter of rats in FCA-induced arthritis. Values are expressed as mean ± SEM of six readings, $^{*}p$ <0.05, $^{**}p$ <0.01, $^{***}p$ <0.001 when compared to arthritic control and $^{\#\#\#}p$ <0.001 when compared to healthy control.

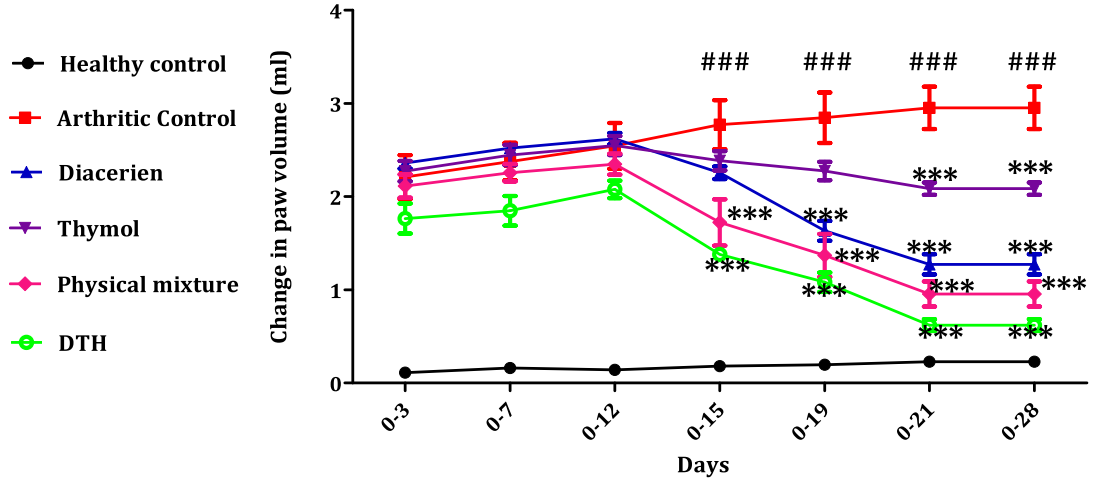

Figure 5. Effect of prodrug on change in left paw volume of rats in FCA-induced arthritis. Values are expressed as mean ± SEM of six readings, $^{*}p$ <0.05, $^{**}p$ <0.01, $^{***}p$ <0.001 when compared to arthritic control and $^{\#\#\#}p$ <0.001 when compared to healthy control.

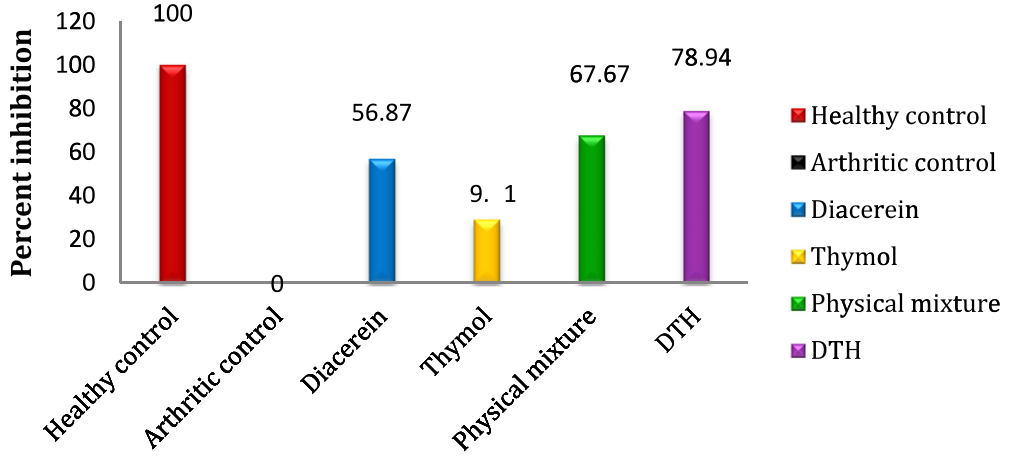

Figure 6. Graph plot showing percent inhibition of edema in FCA induced arthritis model.

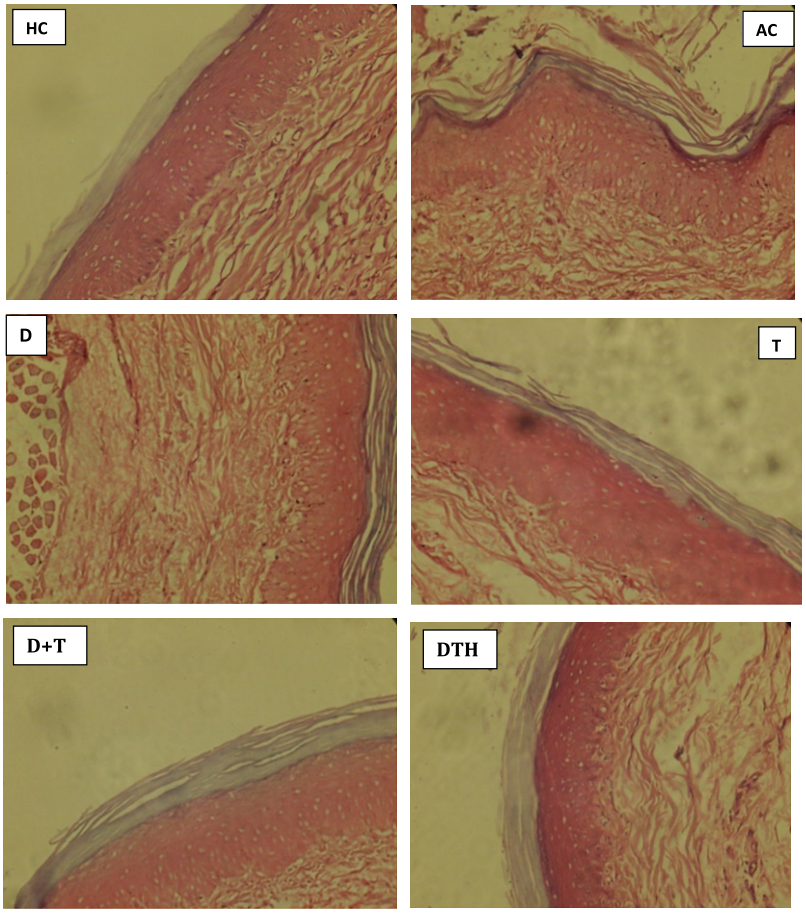

Figure 7. Photomicrographs of histopathological studies of rat ankle joint. Healthy control (HC): No cellular infiltration, no bone necrosis, no connective tissue proliferation, no edema no adjacent tissue involvement. Arthritic control (AC): Cellular infiltration, necrosis of bone, connective tissue proliferation, edema, adjacent tissue involvement. Diacerein (D): Cellular infiltration, necrosis of bone, connective tissue proliferation, edema, adjacent tissue involvement. Thymol (T): Cellular infiltration, necrosis of bone, connective tissue proliferation, edema, adjacent tissue involvement. Physical mixture (D+T): No cellular infiltration, no bone necrosis, no connective tissue proliferation, no edema, no adjacent tissue involvement. DTH: No cellular infiltration, no bone necrosis, no connective tissue proliferation, no edema, no adjacent tissue involvement.

Table 2
Results of ulcerogenic activity

Compounds	Dose (mg/kg)[a]	Ulcer index ± SD[**]
Healthy control	—	1.78 ± 0.60
Diacerein	50	6.03 ± 0.15
Thymol	20.4	1.98 ± 0.70
Physical mixture	50 + 20.4	5.90 ± 0.12
DTH	68	4.17 ± 1.03

[a] Dose 10 times the equimolar dose.
[**] Average of six readings; p <0.01.

confirmed formation of ester linkage. The ^{13}C NMR of DTH exhibited characteristic chemical shifts for aromatic, isopropyl and methyl carbons which also confirmed the structure of DTH.

Stability of DTH was studied in HCl buffer (pH 1.2) and phosphate buffer (pH 7.4) simulating the environment in stomach and small intestine respectively. DTH was stable in HCl buffer (pH 1.2) till 3 h and acid resistant. In phosphate buffer (pH 7.4), hydrolysis of DTH started at around 30 min after introduction with total 90.6% release of diacerein at the end of 4.5 h following first order kinetics. To simulate the enzymatic environment of GIT, release of diacerein from DTH was also studied in stomach and intestinal homogenates of rat that inherently contain esterases which are involved in the hydrolysis of ester linkage. Negligible hydrolysis of DTH was observed in stomach homogenate which is in accordance with the similar behavior in HCl buffer (pH 1.2). However 94.3% release of diacerein was furnished in small intestinal homogenate at the end of 4.5 h which supports hydrolysis of DTH in phosphate buffer (pH 7.4). The rate of hydrolysis of DTH in small intestinal homogenate was more as compared to phosphate buffer (pH 7.4). These results indicate that hydrolysis of ester (DTH) was pH (weakly basic) as well as enzyme (esterase)—mediated.

Statistically significant changes in various parameters were observed thirteen days after administration of 0.1 ml FCA injection into the sub-plantar region of left hind paw. During the course of 28 days, weight in healthy control animals increased owing to regular feed and normal growth while in arthritis control the weight decreased due to full blown development of arthritis. Diacerein, physical mixture (D + T) and DTH increased the body weight near to normal (baseline value) while thymol had a mild effect on body weight.

In arthritic control, joint diameter increased slowly from 3rd day onwards reaching a maximum on 28th day indicating severity of inflammation in joint. Diacerein-treated group showed a gross reduction in joint diameter from 15th day onwards reaching a minimum on 28th, indicating significant anti-inflammatory activity. DTH brought joint diameter almost near the baseline value. Effect of DTH was more pronounced than diacerein, thymol and physical mixture.

Change in paw volume was calculated as a difference between paw volume of each day and paw volume of 0th day. Therefore,

the group showing minimum change indicates maximum anti-inflammatory activity because the minimum change implies that the inflamed/swollen paw was almost brought near to the normal condition. In this respect, DTH exhibited maximum protection against inflammation. Physical mixture, diacerein and thymol also had significant anti-inflammatory effect which was less than DTH. On 28th day, diacerein offered 57% inhibition of inflammation while plain thymol offered 29.4% inhibition, physical mixture offered more protection against inflammation (68%) while the prodrug exhibited highest protective effect in terms of 79% inhibition of edema.

After 28 days study, all animals were sacrificed and samples of ankle joints were sent for histopathological studies. The histopathological evaluation of tibio-tarsal joint of healthy control animal showed unremarkable synovial tissue without any evidence of inflammation or granuloma formation. The section exhibited normal architecture characterized by normal muscle, subcutaneous and synovial tissue. Sections of joints of arthritic control group indicated inflammation and granuloma formation along with inflammatory cell infiltration in the subcutaneous tissue and chronic mononuclear inflammatory cell infiltration in synovial tissue indicating chronic synovitis with subcutaneous inflammation. The impressions observed for diacerein and thymol-treated groups were almost similar with respect to subcutaneous inflammation. However, the former showed moderate synovitis and the latter showed mild synovitis. Animals treated with physical mixture of diacerein and thymol indicated inflammatory cell infiltration in sub-cutaneous tissue and chronic mononuclear inflammatory cell infiltration in synovial tissue. The overall impression was of mild synovitis with subcutaneous inflammation. Animals treated with DTH exhibited no inflammatory cell infiltration in the sub-cutaneous tissue with mild mononuclear cell infiltration in the synovial tissue. The impression was absence of synovitis with subcutaneous inflammation.

The results of ulcerogenic activity (Table 2) revealed that diacerein when directly administered orally in plain form, showed higher ulcer index (6.03 ± 0.15), whereas DTH exhibited lower ulcerogenic potential (ulcer index: 4.17 ± 1.03) which may be ascribed to temporary masking of carboxylic group and stability of prodrug at the acidic pH of stomach.

In conclusion, conjugation of diacerein with thymol increased its lipophilicity, which might be responsible for its better absorption and enhanced bioavailability. The prodrug was stable in acidic pH and stomach homogenates while almost complete release of diacerein was observed (91–94%) in phosphate buffer (pH 7.4) and small intestinal homogenates. Selection of thymol as an antioxidant carrier in mutual prodrug design was justified as the prodrug brought about gross reduction in joint diameter, paw volume and ulcer index and exhibited better anti-inflammatory/anti-arthritic effects than standard drugs and their physical mixture which could be ascribed to positive contribution of thymol. The chronic synovitis induced by FCA in rats was significantly brought back to normal by marked anti-arthritic effect of DTH in comparison to moderate effects of diacerein and physical mixture while thymol showed mild anti-arthritic effect. It can be concluded that combination therapy of disease modifying agent with antioxidant in the form of co-drug could be a promising approach in the effective management of RA.

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33. DTH: mp 256 °C (d; uncorrected), R_f 0.73 (Toluene: methanol; 7:1 v/v) yield 73.54%, Log*P* 0.565, IR (υ, cm^{-1}, KBr): 2918, 2851 (aromatic C–H stretching), 1780 (C=O stretching conjugated ester), 1745(C=O stretching non-conjugated ester), 1701, 1672 (C=O cyclic ketone of anthraquinone), 1311, 1269, 1244 (C–O stretching ester), 1450, 1381(CH_3 bending), 1047–640 (aromatic C–H out of plane bending). ^{1}H NMR (δ, ppm, DMSO-d_6): 1.18–1.29; 2 × CH_3; isopropyl group [d; 6H], 1.71–1.77; 2 × CH_3; acetyloxy group [s; 6H], 2.48; CH_3; benzillic methyl [s, 3H], 3.23–3.35; CH; benzillic methine [m; 1H], 7.35–7.97 ring protons [m, 8H]. ^{13}C NMR (δ, ppm, DMSO-d_6): 122.09–137.36; 12 × C aromatic rings A & C; 191.77, 191.94; 2 × ester carbonyls [non-conjugated], 161.30, 161.60, 161.92; 2 × ketone carbonyls [conjugated] and 1 × ester carbonyl [conjugated], 20.74–25.51; 5 × CH_3, 30.14; CH methine, 115.48–118.27; 6 × C aromatic ring D.
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