ANTI-PLATELET AND ANTI-ARTHRITIC ACTIVITY OF ORTHOCETAMOL (2-ACETAMIDOPHENOL): AN ORTHO (*o*) POSITIONAL ISOMER OF PARACETAMOL

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ABSTRACT

Platelet aggregation plays a pivotal role in the pathogenesis of cardiovascular diseases and rheumatoid arthritis. Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used for the prevention of such diseases. NSAIDs are associated with the severe risk of ulcer and bleeding disorders due to their acetylated chemical structure. Paracetamol is often regarded as NSAIDs. It is a nonacetylated monophenolic drug with mild anti-platelet and anti-inflammatory activity at high dose range which is clinically insignificant.In the current research work we evaluated the effects of 2-acetamidophenol, a positional isomer of paracetamol against *in vitro* human platelet aggregation and collagen induced rheumatoid arthritis model. We used two aggregating agents namely arachidonic acid and adenosine 5'-diphosphate against 2-acetamidophenol. Aggregation was monitored through a dual channel aggregometer. We found that 2-acetamidophenol has several times more potent anti-platelet and anti-arthritic potential than aspirin. Our results demonstrate that 2-acetamidophenol may be a strong drug candidate for the prevention of cardiovascular diseases and rheumatoid arthritis.

Key words:

INTRODUCTION

Platelet aggregation is one of the leading factors in the progression of cardiovascular diseases (CVDs) and rheumatoid arthritis (R.A) (1). Nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin and aspirin-like drugs possess anti-inflammatory and anti-platelet potential. Aspirin is used as the drug of choice for the prophylaxis of most of CVDs and R.A (2). It is well known that prolonged use of aspirin and aspirin-like drugs are associated with severe risks of gastrointestinal adverse effects (3). Recent studies have shown that NSAIDs-induced CVDs are rapidly increasing around the world (4).Therefore there is a great need of novel, safe, and effective anti-platelet and cardio protective drugs.

Phenolic compounds are well known cardio protective agents due to their potent antioxidant potential (5). Paracetamol is a simple monophenolic compound with mild anti-platelet activity. The antioxidant properties(6)and non-ulcerative nature of paracetamol is well documented in the available literature (7). Paracetamol is often regarded as an NSAID, but it is not clinically used as substitute of NSAIDs for prophylaxis of CVDs due to its weak anti-platelet potential. However; Paracetamol synergies the pharmacological actions of NSAIDs. Paracetamol indirectly inhibits Cyclooxygenase (COX) and is involved in the reduction of oxidative stress (8).Paracetamol modulates prostaglandin biosynthesis by inhibiting prostaglandin H synthase and shows little anti-inflammatory effects (9).Paracetamol anti-platelet potential is rarely documented in literature.

Platelet aggregation may be stimulated by several aggregating agents including arachidonic acid and Adenosine 5'-phosphate (ADP) (10). It is known that most of the aggregating agents stimulate the aggregation via formation of the Thromboxane A2(TXA2) from arachidonic acid metabolism; this effect may be inhibited by aspirin and aspirin-like drugs (11). Several inflammatory mediators are common in RA and CVDs.Several monophenolic and polyphenolic compounds have been reported for reducing consequences of inflammation, platelet aggregation and antioxidant activity (12). In the current research work, 2-acetamidophenol (2-AMP); a monophenolic positional isomer of paracetamol also called orthocetamol has been observed for anti-arthritic and anti-platelet activity.2-AMP is a commercially available compound and is also known as, O-hydroxyacetanilide, 2-hydroxyacetamide, O-acetaminophenol and 2-acetylaminophenol. In our study, we found that 2-AMP has a distinctive anti-platelet profile in addition to anti-arthritic potential at significantly lower doses than those of aspirin.

MATERIALS AND METHODS

Chemicals and solutions

All organic solvents, chemicals and reagents used in the experiments were of analytical or grade. Most were purchased from Sigma chemical company (U.S.A), and other leading suppliers. ADP was dissolved in saline to obtain the final concentration of 5 µM. For the preparation of stock concentration of 5 mM aspirin, 0.9mg of aspirin was dissolved in 50 µL of DMSO and it vortexed for 1 minute. Then 950µL of phosphate buffer saline (PBS) was added to make up the volume up to 1000 µL or 1 mL of aspirin. 0.6mg of paracetamol was dissolved in 50µl of DMSO and it vortexed for 1 minute, and then 950 µl of PBS was added to give the stock concentration of 4 mM. 2-acetamidophenol was prepared just like paracetamol. In order to make stock solution of 43.3 mM of 0.2% w/v of aqueous sodium carbonate, it was flushed with oxygen-free nitrogen for one minute and then was stored in aliquots at -20 ^{0}C. 10 µl of stock solution of arachidonic acid was used to induce optimal platelet aggregation.

The selection of animals

Female Sprague Dawley rats, with body weight 220-250 g, were used in this study. Animal were procured from animal house of Dr Panjwani Centre for Molecular Medicine and Drug Research. The rats were housed at 25 C and on a 12-h light/dark cycle with free access to laboratory rat food pellets and water. 8 animals per group for each dose were used. The specifications given in Helsinki Resolution 1964 were followed during animal handling. This study was ethically approved by ethical review committee for pre clinical studies from University of Karachi Pakistan.

Human platelet aggregation studies

Preparation of human platelets

Venous blood was taken from healthy human subjects of either sex, aged (25 ± 4) years, and a body weight of 60 ± 7 Kg. Subjects were reported to be free of medication for one week. Blood samples were mixed with 3.8 % (w/v) sodium citrate solution (9:1) and were centrifuged at 260×gfor 15 min at 20 ^{0}C to obtain platelet rich plasma (PRP), and at 1200×g for 10 min to obtain platelet poor

plasma (PPP). Aggregation studies were carried out at 37 ^{0}C and PRP platelet counts were between 2.5 and 3.0×10^9/l of plasma. All experiments were performed within 3 hours of PRP preparation (9).

Measurement of platelet aggregation

Aggregation was monitored using dual channel lumi-aggregometer(Lumi-Aggregometer Chronolog VS-400 USA) using 0.45 mL aliquots of PRP. The final volume was increased to 0.5 mL and the test drug dissolved either in normal saline or an appropriate vehicle known to be ineffective on aggregation. Aggregation was induced with adenosine-5' diphosphate (ADP = 5μM) and arachidonic acid (AA= 0.75mM). The resulting aggregation was recorded for 5 min after challenge with the inducing agent by the change in light transmission as a function of time. As the anti-platelet activity of various inhibitors against agonist was established, the dose responded.

Anti-arthritic activity

Induction of arthritis

Collagen was used to induce arthritis. Arthritis was induced as described in the literature (12).Collagen was injected intradermal at the base of the tail using a sterile hypodermic needle under anesthesia using combination of ketamine / xylazine in the dose of 20mg/kg / 5mg/kg. Treatment was initiated on the same day as arthritis induction. The reference drug was indomethacin and paracetamol positional isomer (2-acetamidophenol). The vehicle and drugs were administered intraperitoneally. In addition, control rats were administered only with the vehicle concurrently (13).

Clinical assessment of collagen-induced arthritis (CIA)

Rats were evaluated on alternate days for arthritis using macroscopic scoring system, where 0 = no signs of arthritis, 1 = swelling and/or redness of the paw or 1 digit, 2 = 2 joints involved, 3 = more than 2 joints involved, and 4 = severe arthritis of the entire paw. The arthritis severity score for each rat was calculated by adding the scores of each individual paw (13)

Measurement of hind paws hyperalgesia and edema

The tendency of normal (naive) control and arthritic rats to vocalize following flexion of the tarsotibial joints of both hind paws were tested daily for 22 days starting from day 0. Hyperalgesia is reported as the mean ± SEM number of vocalizations following five lexions of the hind limb tarso-tibial joints, resulting in maximal hyperalgesia (value = 1) when five vocalizations were obtained following five flexions of the paws. Clinical severity of arthritis was also determined by quantifying the change in the paw volume (as an indicator of edema) with a plethysmometer following the hyperalgesia test. The advantage of using this method over diameter measurements of tibiotarsal joint is that it measures the limb in three-dimensions and therefore takes into account any variation of the swelling pattern of individual limbs. The volume of a hind paw is reported as the mean ± SEM in ml. All measurements were made at the same time of day. The body weight and hind paw volumes were measured in both the control and the test groups on days 0 and then on alternate days until day 22 when the experiment ended.

Gait analysis

Locomotion was recorded in test and control groups in the beginning of an experiment and was used as the baseline reading (day 0). Apparatus used for this purpose was the tread scan system (Treadmill Tread Scan System,Clever Sys. Inc Reston, Virginia).This system records a video of animal (mouse or rat) running on a transparent treadmill as input. A mirror is placed at an angle of 45° below the belt section of the chamber, which allows viewing of the floor/paw contact. The video essentially captures the footprints of the animal during exercises on the treadmill. The software provided with this system (tread scan) can analyze the video, and determine various characteristic parameters that are related to the pathophysiological conditions. The parameters measured in this study include the stance time (paw in contact with the floor), the swing time paw in the air, stride length, and running speed (14).

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) software was used to analyze the data. Throughout this study mean ± SEM of means were used to describe the data in figures. The data were analyzed using two-way analysis of variance (ANOVA).

RESULTS

Platelet aggregation

Arachidonic acid and ADP reliably induced platelet aggregation in PRP. 2-acetamidophenol showed very significant activity against arachidonic acid induced platelet aggregation. 1, 5, 50, and 100 µM concentrations of 2-acetamidophenol were tested in this method. Interestingly, it produced the strongest response at very low dose, whereas weakest response at high dose**(Fig.1A).**

ARACHIDONIC ACID

ASPIRIN

DICLOFENAC

Fig.1 A

% AGGREGATION

PARACETAMOL
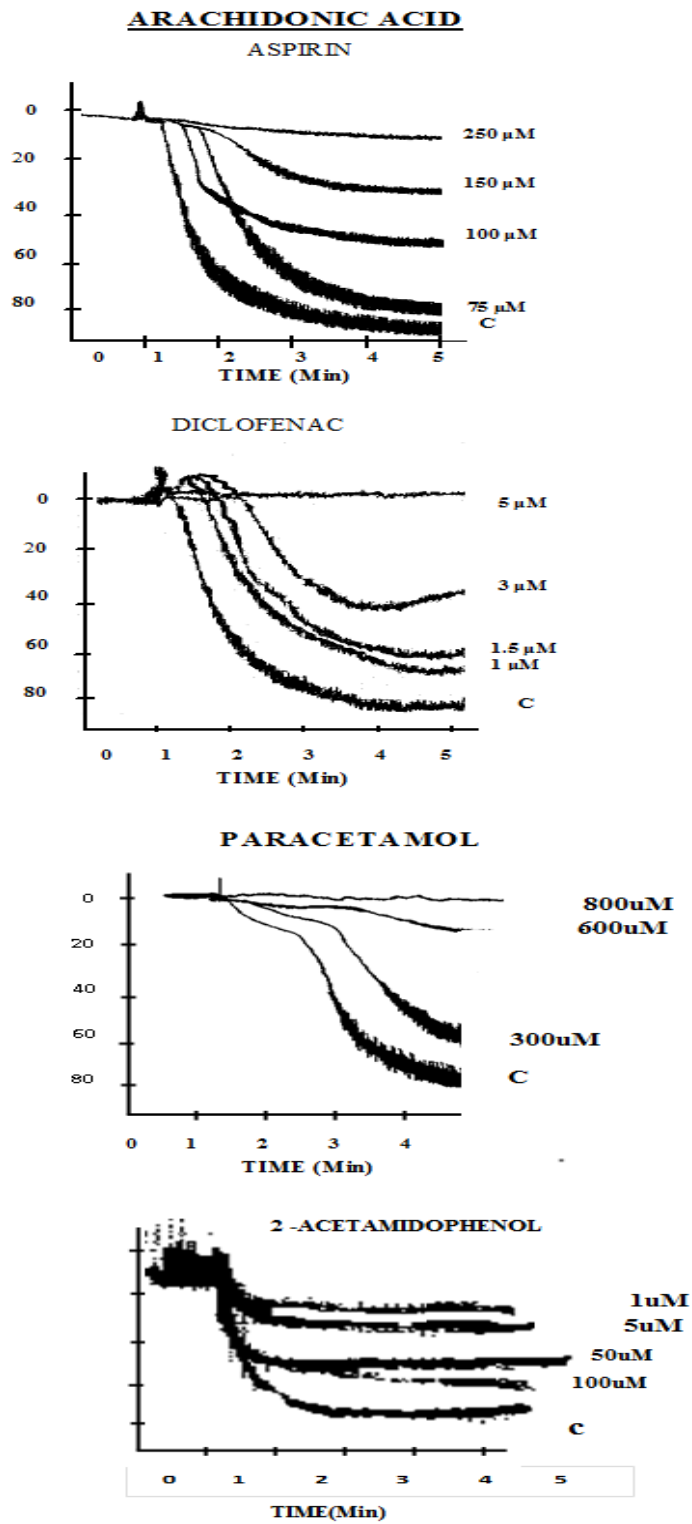
2 -ACETAMIDOPHENOL

2-acetamidophenol at 1 μM reduced 93.8 ± 2.9% platelet aggregation with respect to control. With increasing concentrations, its response was reduced. Finally at 100μM it suppressed only 32.2 ± 2.6% platelet aggregation. When standard drug aspirin was tested, it caused a complete inhibition of arachidonic acid-induced platelet aggregation at 200 μMconcentration**(Table 1)**.

2-acetamidophenol was also more potent than aspirin in the ADP-induced platelet aggregation method. 2-acetamidophenol was ineffective at 1 and 5 μMconcentrations; whereas at 50 μM it inhibited 52 ± 1.4% ADP-induced platelet aggregation. Likewise, its effect against arachidonic acid induced platelet aggregation. With increasing dose, its effect also went down against ADP-induced platelet aggregation. At 100 μM, it showed 39 ± 3.7% inhibition; and at 300μM, it showed 24 ± 1.9% inhibition **(Fig.1B and Table 1).** Aspirin at 200μM concentration only inhibited 31 ± 3.6% ADP-induced platelet aggregation**(Table 1)**.

Table 1: Effect of 2-acetamidophenol and aspirin on arachidonic acid (AA) and adenosine-5'-diphosphate (ADP) induced platelet aggregation.

Compound	Concentration (uM)	Arachidonic acid ± SEM	ADP ± SEM
2-acetamidophenol	1	93.8±2.9	NE
	5	71.2±1.8	NE
	50	51.7±3.1	52 ± 1.4
	10	32.2±2.6	39 ± 3.7
	300	NT	24 ± 1.9
Aspirin	200	100±0.0	31±3.6

n = 7 for each concentration

Values are shown in percentage inhibition (mean ± SEM) of platelet aggregation with respect to control.

NE = Not effective

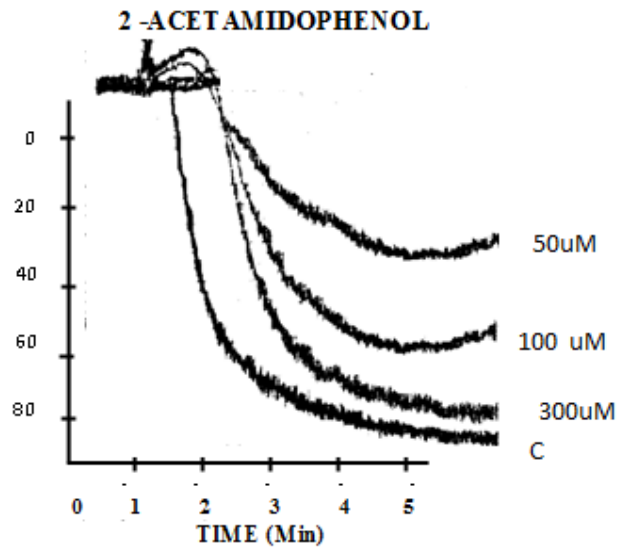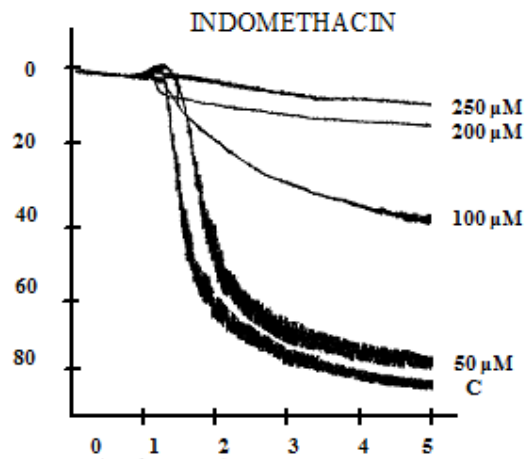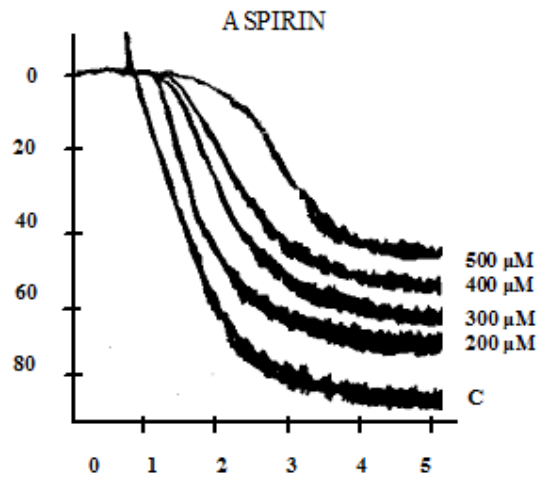NT= Not tested

ADENOSINE-5'-DIPHOSPHATE

ASPIRIN

500 µM
400 µM
300 µM
200 µM
C

Fig.1 B

INDOMETHACIN

250 µM
200 µM
100 µM
50 µM
C

% AGGREGATION

2 -ACETAMIDOPHENOL

50uM
100 uM
300uM
C

TIME (Min)

Antiarthritic activity

Collagen-induced arthritis is one of the recommended models for the induction of arthritis in rats. Method was followed as described inliterature (12). After 10 days of induction of arthritis, animals began to show evidence of clinical inflammation in one or both joints followed by involvement of the metatarsal and interphalangeal joints. The typical time course of development and progression of the disease was assessed by mean arthritis severity score and paw volume, as shown in **Figure 2**. The signs of an arthritic score 3 in untreated arthritic rats treated with only saline and 2-acetamidophenol were evident at day 10. However, the arthritic rats treated with aspirin and indomethacin showed score 2 on day 10. The disease was progressive, with joint recruitment following the same pattern: tarsal, meta phalange, and then interphalangeal. In the vehicle and non-treated arthritic group the incidence was 100% (i.e. All animals in the group were affected) at day 12 and remained such throughout the duration of the experiment. In contrast, treatment with 2-acetamidophenol, indomethacin, and aspirin exerted a significant attenuation in the incidence of CIA; 80% with 2-acetamidophenol, 40% with aspirin treatment, and 70% with indomethacin **(Fig.3).** A large increase was observed in the hind paw volume of untreated, saline-treated rats compared to non-arthritic rats. ANOVA performed over all data showed that this increase in the paw volumes became significantly different from non arthritic rats on day 10 onwards ($P \leq 0.006$). It was observed that the arthritic rats treated with 2-acetamidophenol showed significant decreased in their paw volume compared to controls from days 8-12. In contrast, arthritic rats treated with aspirin and indomethacin showed a gradual, but insignificant increase in their paw volumes until the end of the experiment**(Fig.3).** The body weights of tested animals were not significantly different between the groups before commencement of the study. In the first six days the increment in body weight was similar in all groups and no significant differences were seen between them. However, after day 8, a gradual loss in body weight was observed**(Fig.4)** which became significant on day 10 for untreated, saline treated, and 2-acetamidophenol treated arthritic rats as compared to normal control. This weight loss was consistent until the end of the study **(Fig.4).**Neither the speed nor the velocity of the non-arthritic rat showed much variation over the 22 day duration of the experiment. Both the arthritic rats that were untreated and treated with saline remained unaffected by drug treatment. This was statistically significant on day 6 ($P \leq 0.004$). On the other hand, the 2-acetamidophenol, aspirin, and indomethacin treatments of the arthritic rats showed an increase in their speed from day 2 onwards **(Fig.5)**.
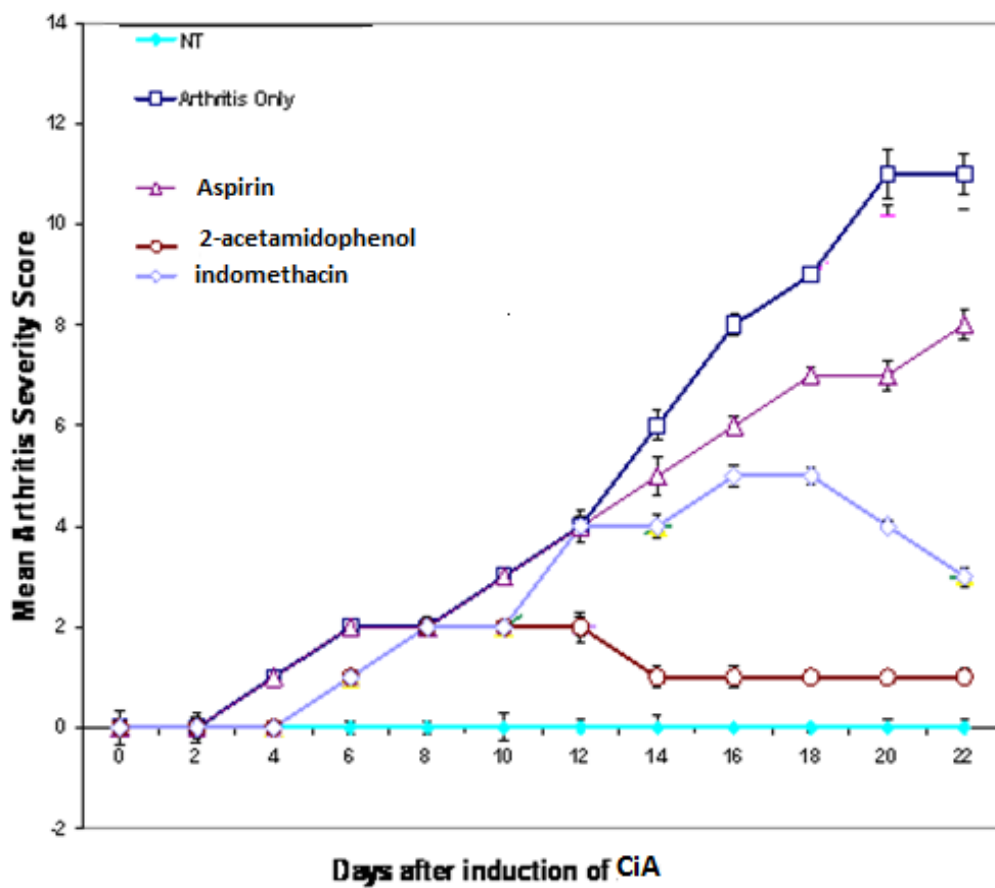
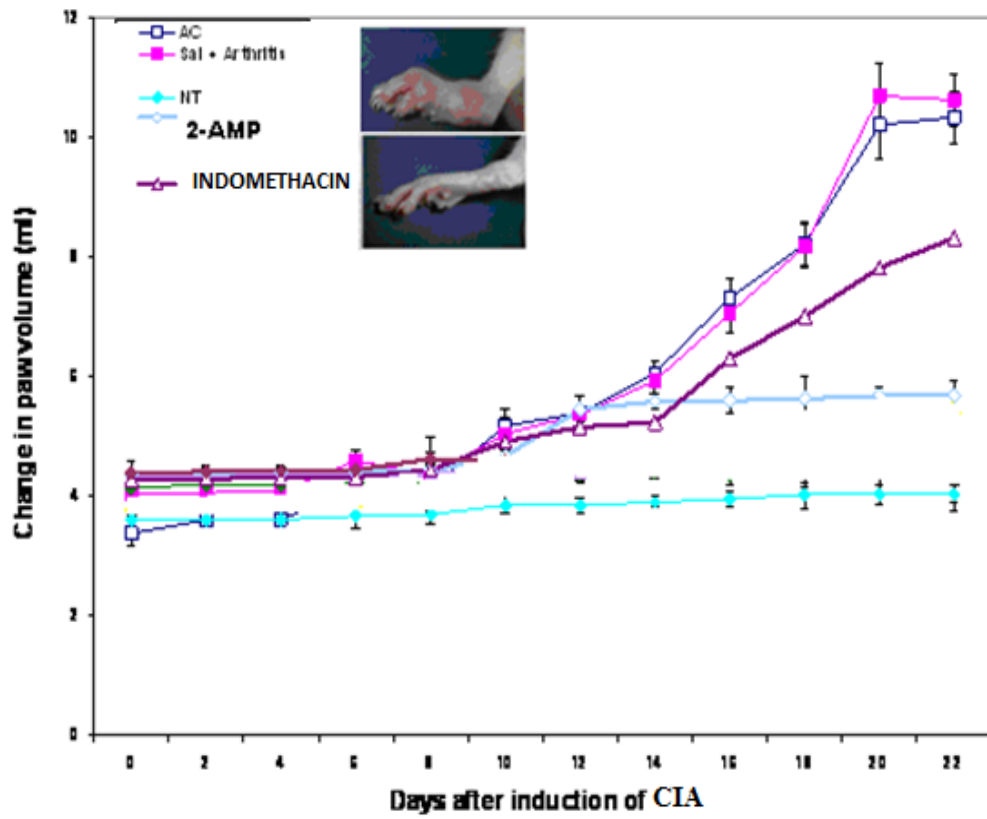**Fig.2**:Arthritis Severity Scores in rats during development of CIA. Effect of NSAIDs and 2-acetamidophenol

Fig.3: Time course of change in hind paw volume after the induction of arthritis. Results are expressed as means ± SE (n = 6)..
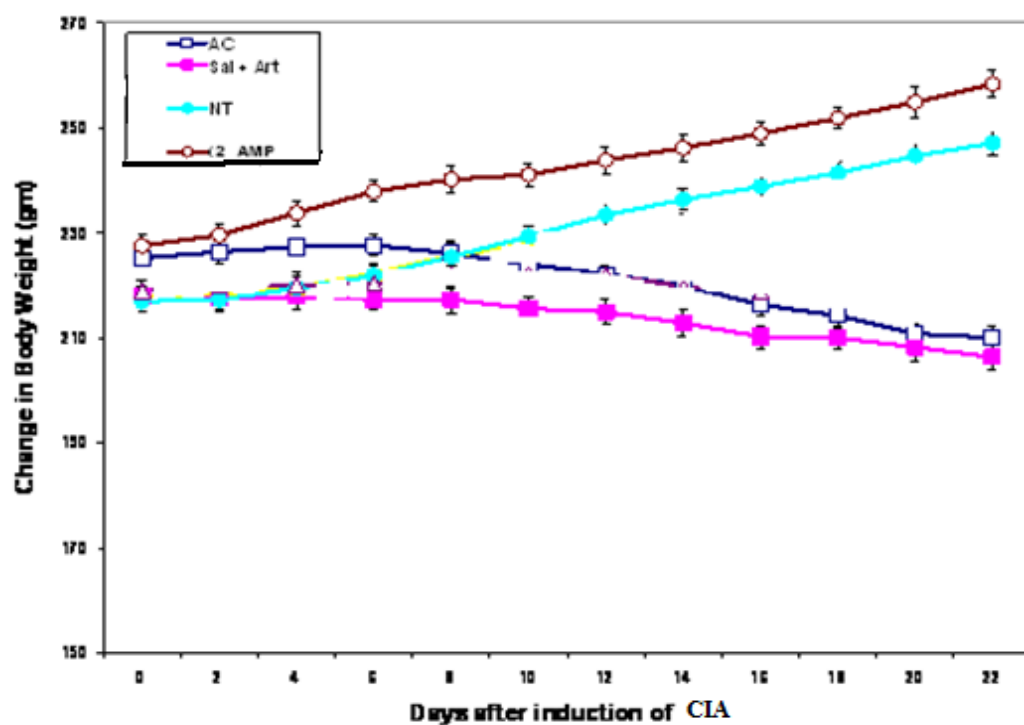
FIg.4:Time course of body weight change in rats with Collagen-induced arthritis Effect of NSAIDS and 2-acetamidophenol on the body weight changes of the arthritic rats has been measured over a period of 22 days.

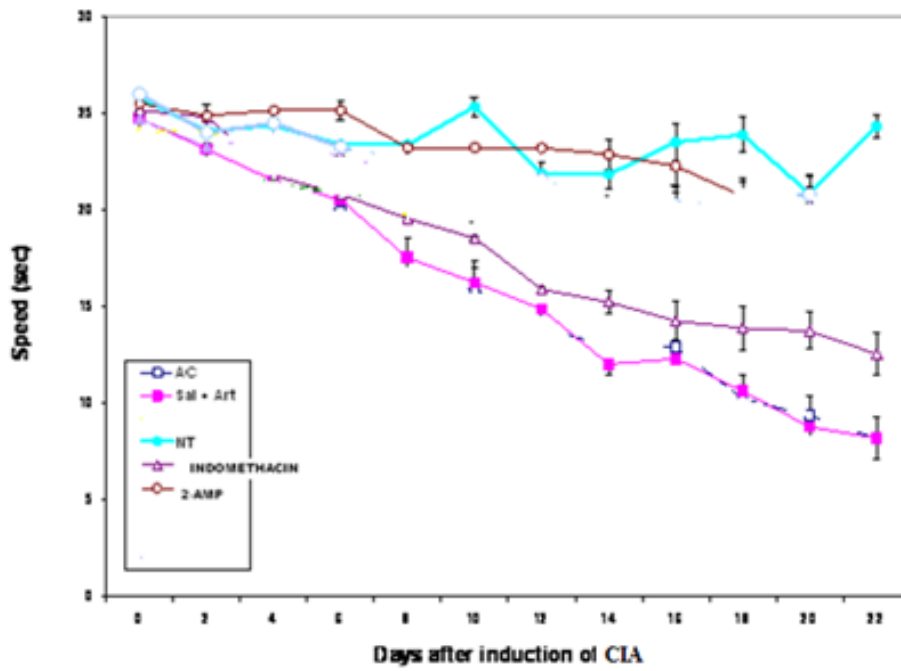**Fig. 5** Time course of the change in walking speed after the induction of arthritis compared with normal rats, 2 –acetamidophenol (2-AMP) and indomethacin-acetamidophenol. Results are expressed as means ± SE (n = 6).

DISCUSSION

Platelet aggregation aggravates the clinical manifestation of arterial thrombotic disease. NSAIDs are commonly used to prevent the platelet aggregation, but these drugs have side effects and patients become resistant on prolong use (15). Therefore, it is important to develop the new anti-platelet aggregating compound with a better efficacy and safety profile than those of existing standard drugs. In this context, we investigated *in vitro* effects of 2-acetamidophenol, an ortho (*O*) positional isomer of paracetamol, on human platelet aggregation. The platelet responses are distinguishable on the basis of requirement for different concentrations of the aggregating agents; these aggregating agents are arachidonic acid, ADP, epinephrine, nor epinephrine, vasopressin and serotonin (16). TXA_2, formed from liberated arachidonic acid, not only produced aggregation and change of shape, but also released the contents of dense granules. The aggregation triggered with ADP or epinephrine is mainly independent of COX activity and TXA_2 release. ADP and epinephrine bind directly to their own receptors on the surface of the platelet. Norepinephrine, vasopressin, and serotonin are weak activators capable of triggering only shape change and aggregation (17).NSAIDs produce their inhibitory effects on aggregation via modulation in arachidonic acid metabolism and thereby interfering with the production of TXA_2 and TXB_2, necessary for platelet aggregation (18).When we tested 2-acetamidophenol against arachidonic acid-induced platelet aggregation, we found significant anti-platelet aggregating effects of this compound at lower doses. Based on our findings in arachidonic acid-induced platelet aggregation assay, we suggest that 2-acetamidophenol inhibited platelet aggregation via modulation in arachidonic acid metabolism, however, anti-platelet mechanism of 2-acetamidophenol needs further investigation.

ADP is an important secondary mediator of platelet activation. It is commonly used to induce the platelet aggregation. It initiates aggregation, which to an extent is independent of COX-thromboxane pathway.This agonist activates G protein coupled receptors, fibrinogen receptor, and clot formation. Second messengers such as Ca^{2+}, protein kinase C, and tyrosine kinase increase platelet aggregation, whereas cAMP inhibits it. G_1 acts by decreasing cAMP level and leads to platelet aggregation. Activation of GQ enhances the cytosolic Ca^{2+} concentration by stimulating inositol triphosphate (IP^3) formation through the inositol pathway that leads to further activation of platelet aggregation (19). We found that 2-acetamidophenol exhibits less sensitivity against ADP-induced platelet aggregation as compared to arachidonic acid-induced aggregation. At 50 μM, it showed only $52 \pm 1.4\%$ inhibition. However, it was found to be more potent and more effective

than aspirin against ADP-induced platelet aggregation, which blocked only $31 \pm 3.6\%$ platelet aggregation at 200 µM.Paracetamol is often regarded as an NSAID, *in vitro* it shows weak inhibition of COX-1 and less release of TXB_2 as compared to conventional NSAIDs (20). COX-2 and COX-3 inhibitory potential of paracetamol is also reported in the literature (21). Paracetamol is believed to be clinically ineffective in the prophylaxis of atherosclerosis. It has been suggested that it does not have significant anti-platelet and anti-inflammatory effects (22).Munsterhjelm and coworkers reported the anti-platelet activity of paracetamol at high doses. 2-acetamidophenol was found much potent than paracetamol. Thus 2-actamidophenol may not only provide better efficacy, but also offer a better safety profile than conventional NSAIDs.

We describe a novel approach to quantify the hyperalgesia in a rat model of rheumatoid arthritis using gait analysis. It has previously been reported that gait changes in the arthritic rat can be used as an objective measure of chronic. The principal aim of this study was to examine the effect of prolonged administration of low doses of 2-acetamidophenol on the progression of hind-paw inflammation, disease progression, and gait analysis. A strong correlation was found between parameters obtained from gait analysis and the disease progression in the collagen induced arthritic rats. The administration of our results suggests that a prolonged administration of low doses of these 2-acetamidophenol (1mg/kg) is effective in preventing the development of chronic pain which, once established, is difficult to treat with conventional analgesics. 2-acetamidophenol also had a significant effect on disease progression as measured by no further weight loss as observed in the untreated or saline-treated collagen-induced arthritis. They also significantly reduced the joint inflammation like indomethacin when administered for a prolonged time at low doses. 2-actamidophenol and indomethacin-treated arthritic rats showed a marked decrease in the vocalization compared to arthritic control animals. We analyzed 2-acetamidophenol for antiarthritic potential in the collagen-induced arthritis model as described by literature (23). We found potent antiarthritic activity of 2-acetanidophenol compared to indomethacin. With the same pattern, the compound was only active at lower dose ranges (1mg/kg – 5mg/kg) and was ineffective in higher doses. Finally, we tested the arthritic rat and 2-acetamidophenol treated rat in the gait analysis model. The possible mechanism of its potential to inhibit arthritic scores, the inhibition of release of tumor necrosis factor-alpha (TNF-α) might be involve. TNF-α is found to be a leading molecular target in rheumatoid arthritis. Its blockers are available for the management of the consequence of rheumatoid arthritis (13). On the other hand, 2-acetamidophenol is reported as TNF-α blocker (24). We found 2-acetamidophenol with significant anti-rheumatic potential in the collagen-induced rat

paw edema model. Previously, 2-acetamidophenol showed potent anti-rheumatic potential in adjuvant induced rat paw edema model (25).

Conclusively, due to phenolic nature of 2-acetamidophenol, it doesn't have ulcerative characteristics (Data is not shown) as commonly found in classical NSAIDs. The search for an ideal novel non-ulcerative NSAID has remained a primary target for researchers and medical scientists. NSAID-induced toxicities and its complications are a bigger challenge in the treatment of chronic inflammatory diseases such as rheumatoid arthritis and atherosclerosis. Our results indicate that 2-acetamidophenol might be the best drug candidate for the treatment of rheumatoid arthritis and atherosclerosis with minimal or no risk of gastric ulcer.

REFERENCES

1. Gasparyan AY, Stavropoulos-Kalinoglou A, Mikhailidis DP, Douglas KM, Kitas GD. Platelet Function in Rheumatoid Arthritis: Arthritic and Cardiovascular Implications, Rheumatol Int 31, 153-64, 2011.

2. Capone ML, Tacconelli S, Di Francesco L, Sacchetti A, Sciulli MG, Patrignani P. Pharmacodynamics of Cyclooxygenase inhibitors in humans, Prostaglandins Other Lipid Mediate 82, 85-94, 2007.

3. Allison MC, Howatson AG, Torrance CJ, Lee FD, Russell R.I. Gastrointestinal damage associated with the use of no steroidal anti-inflammatory drugs, N Engl J Med 327, 749-54, 1992.

4. Hermann M. Cardiovascular risk associated with non-steroidal anti-inflammatory drugs, Curr Rheumatic Rep 11, 31-35, 2009.

5. Lecour S, Lamont KT. Natural polyphenols and cardio protection, Mini Rev Med Chem 11, 1191-99, 2011.

6. Tripathy D, Grammas P. Acetaminophen inhibits neuronal inflammation and protects neurons from oxidative stress, J Neuroinflammation6, 10, 2009.

7. Galunska B, Marazova K, Tankova T, Popov A, Frangov P, Krushkov I, Di Massa A. Effects of paracetamol and propacetamol on gastric mucosal damage and gastric lipid

peroxidation caused by acetylsalicylic acid (ASA) in rats, Pharmacol Res 46, 141-47, 2002.

8. Graham GG, Scott KF. Mechanism of action of paracetamol, Am J Ther 12, 46-55, 2005.

9. Robak J, Kostka-Trabka E, Duniec Z. The influence of three prostaglandin biosynthesis stimulators on carrageenan induced edema of rat paw, Biochem Pharmacol 29, 1863-65, 1980.

10. Saeed SA, Ahmad N, Ahmed S. Dual inhibition of cyclooxygenase and lipoxygenase by human haptoglobin: its polymorphism and relation to hemoglobin binding, Biochem Biophys Res Commun 353, 915-20, 2007.

11. Botting RM. Inhibitors of cyclooxygenases: mechanisms, selectivity and uses, J Physiol Pharmacol 57, 113-24, 2006.

12. Biesalski HK. Polyphenols and inflammation: basic interactions, Curr Opin Clin Nutr Metab Care 10, 97-107, 2007.

13. Hamer ER, Apfel MI, Carvalho JJ, Pereira MJ, Levy RA. Evaluation of cholesterolinfluence in type II collagen–induced arthritis in DBA/1J mice: an auto radiographic study, J Cell Mol Med 6, 407-14, 2002.

14. Simjee SU,Pleavry BJ, Coulthard P. Modulation of the gait deficit in arthritic rats by infusion of muscimal and bicuculine, Pain 109, 453-460, 2004.

15. Jawed H, Shah SU, Jamal S, Simjee SU. N-(2-hydroxy phenyl) acetamide inhibits inflammation-related cytokines and ROS in adjuvant-induced arthritic (AIA) rats, Int Immunopharmacol 10, 900-905, 2010.

16. Patrono C. Aspirin resistance: definition, mechanisms and clinical read-out, J Thromb haemost 1, 1710-13, 2003.

17. Blockmans D, Deckmyn H, Vermylen J. Platelet activation, Blood Rev 9, 143-56, 1995.

18. Paul BZ, Jin J, Kunapuli SP. Molecular mechanism of thromboxane A (2)-induced platelet aggregation. Essential role for p2t (ac) and alpha (2a) receptors, J Biol Chem 274, 29108-

29114, 1999.

19. Moncada S, Vane JR. Arachidonic acid metabolites and the interactions between platelets and blood-vessel walls, N Engl J Med 1142-47, 1979.

20. Offermanns S, Toombs CF, Hu YH, Simon MI. Defective platelet activation in G alpha(q)-deficient mice, Nature 389, 183-86, 1997.

21. Mitchell JA, Akarasereenont P, Thiemermann C, Flower RJ, Vane JR. Selectivity of non steroidal anti-inflammatory drugs as inhibitor of constitutive and inducible cyclooxygenase, Proc Natl acad Sci USA 90, 11693-11697, 1993.

22. Schwab JM, Schluesener HJ, Laufer S. COX-3: just another COX or the solitary elusive target of paracetamol? Lancet 361, 981-82, 2003.

23. Bertolini A, Ferrari A, Ottani A, Guerzoni S, Tacchi R, Leone S. Paracetamol: new vistas of an old drug, CNS Drug Rev 12, 250-75, 2006.

24. Goekoop YP, Allaart CF, Breedveld FC, Dijkmans BA. Combination therapy in rheumatoid arthritis. Curr Opin Rheumato,13, 177-83, 2001.

25. Yoon JH, Baek SJ. Molecular targets of dietary polyphenols with anti-inflammatory properties, Yonsei Med J 46, 585-96, 2005.