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THE PHARMACOKINETIC STUDY OF ASPIRIN, PARACETAMOL AND NAPROXEN WITH MAGNESIUM SULFATE

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ABSTRACT
The present study was designed to evaluate the bioavailability of single drug as well as drug with Mg(II) complexes in the systemic circulation of rats. 132 healthy rats were selected for this study. The rats were fasted for 12 hours (overnight) prior to drug administration and kept fasting up to blood collection after administration of the drugs. The rats were divided in to three groups for each drug of study: control (rats without giving any drugs for each analysis) and group 1 for reference (single drug) and group 2 for test drug i.e. drug-Mg complexes. In this study both the test (drug-Mg complex) drug and corresponding reference drug were administered at the dose of aspirin at 10 mg/kg body weight, paracetamol at 16 mg/kg body weight and naproxen at 16 mg/kg body weight in the solution form through oral route. From the pharmacokinetic study, it was found that concomitant administration of magnesium with aspirin slightly increased elimination rate and lowered the bioavailability than the reference aspirin. When magnesium was concomitantly administered with paracetamol, it markedly increased elimination rate and decreased the bioavailability than that of the reference paracetamol. When magnesium was concomitantly administered with naproxen, it decreased elimination rate and remained in the systemic circulation for longer time.

KEYWORDS: Bioavailability, Paracetamol, Aspirin, Magnesium Sulfate, NSAID.

INTRODUCTION
The biological activities of the drug complexes may affect the stability of the systems and their therapeutic actions via changing pharmacokinetic and pharmacodynamics parameters.Several organometallic compounds are containing remarkable pharmacological effects and are utilized as active ingredients. Magnesium is present in three different states in the biological system: freely coordinated to water, in association with, anions and attached to protein. On average, the human body is made up of approximately 24g of magnesium. Several other drugs are being tested to find other uses of magnesium in drugs.[1] Magnesium cation (Mg++) is the second most abundant intracellular cation which has been proven to possess important roles in the human body. Along with its contribution to energy production through Adenosine Triphosphate (ATP), it contributes to the maintenance of serum sodium, calcium, potassium, smooth muscle tone in the vessel wall, it is necessary for normal neuromuscular function and Ca++ and K+ transportation across the plasma membrane.[2] A deficiency of Mg in the human body is linked with an increase in risks like cardiovascular diseases and thermogenesis, like an increase in oxidative stress, cytokine synthesis, synthesis of nitric oxide and mediators of inflammation, and adhesion molecules on microvascular endothelial cells.[3] The suggested curative properties of Mg-based non-steroidal anti-inflammatory drugs (NSAIDs) sometimes lead to a development in some Mg (ll) complexes of NSAIDs with an enhancement in the anti-inflammatory actions and reduction in gastrointestinal toxicity when compared with its parent drug. For all of these reasons, the complexion of aspirin, paracetamol, and naproxen with metal ions is a blooming field of research. Most of the NSAIDs are weak organic acids that are absorbed into the body efficiently after an oral administration. However, food affects the oral absorption of some NSAIDs (phenylbutazone, meclofenamate, flunixin, and robenacoxib). Different NSAIDs are also found in their parental formulation for intravenous, intramuscular, and subcutaneous monitoring. These parental formulations

are alkaline in nature (phenylbutazone) and may lead to tissue necrosis if injected perivascularly. After absorption, most NSAIDs are extensively (up to 99%) attached to plasma proteins, with a small proportion of unbound drug left to be active in the tissues. NSAIDs can also compete for binding sites with other high protein bound compounds, which causes drug displacement, where the displacement has very negligible therapeutic activity as it does not affect the concentration of the free drug. Because NSAIDs are heavily protein-bound and extravasation of protein takes place in inflammation, NSAIDs tend to increase concentration in areas of inflammation. In addition, it hasthe duration of action always exceeds that predicted by the elimination of half-life.[4] Aspirin is easily absorbed from the stomach into the intestine by passive diffusion. Salicylate distributes fast into the fluid compartments. It forms bonds with albumin in the plasma. Paracetamol is also easily absorbed into the gastrointestinal tract. It is homogeneously distributed in the body fluids.[5] Naproxen is also absorbed from the gastrointestinal tract. Its peak plasma concentrations are obtained 2-4 hours after intake. Naproxen is absorbed from the gastrointestinal tract with vivo bioavailability of about 95%. Naproxen is highly bound to plasma, responsible for about 99.6% of the total plasma level of 23-40ug/ml.[6] The objective of the research was to study the pharmacokinetic profile and bioavailability of the drugs with Mg (ll) complexes in a systematic circulation of the rat through areas under curve (AUC) calculation. Its anti-inflammatory actions and other possible toxic effects with these drugs were also studied.

MATERIALS AND METHODS

MATERIALS

Aspirin and paracetamol were collected from Square Pharmaceuticals Ltd., Dhaka, Bangladesh; naproxen was collected from SK+F Pharmaceuticals Ltd., Dhaka, Bangladesh. Magnesium sulfate heptahydrate (E. Merk, India, Ltd.), Methanol (Active Fine Chemicals Ltd., Dhaka, Bangladesh), Demineralized water and other reagents were supplied from laboratory.

INSTRUMENTS

Centrifuge Machine, HPLC (Shimadzu, Japan).divided into three groups having five rats in each group for each drug of study: control (rats without giving any drugs for each analysis) and group 1 for reference (single) drug and group 2 for test sample (drug-Mg complexes). The amount of dose was given to the rat by considering average body weight 240-260 g. The dose of drug (both reference and test) was given in a specific time interval. After collection of blood, they were centrifuged and serums were collected and preserved in vials at 4oC. Then the concentrations (µg/mL) of drug and drug-Mg complexes in serum were analyzed by HPLC.

DOSAGE AND DRUG ADMINISTRATION

All the drugs were used in the solution form. Aspirin (both test and reference drugs) at a dose of 10 mg/kg body weight, paracetamol (both test and reference drugs) at a dose of 16 mg/kg body weight and naproxen (both test and reference drugs) at a dose of 16 mg/kg body weight were administered to the rats of specific group by oral route. Drug-Mg complexes were administered concomitantly at a dose of 10 mg/kg in the rats of specific group (group 2) by oral route.

EXPERIMENTAL ANIMAL

Young Swiss-albino rats, average weight range 240-260 g were used for the experiment. The rats were purchased from the animal house of the Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh. They were kept in standard environmental conditions in the Animal House, Institute of Food and Nutrition Sciences (IFNS), University of Dhaka, Bangladesh for one week for adaptation after their purchase and fed ICDDR, B formulated rodent food and water ad libitum. The experiment was carried out in the Department of Pharmacy, State University of Bangladesh, Dhanmondi, and Dhaka, Bangladesh.

METHOD

The Pharmacokinetic analysis was carried out in the Department of Pharmaceutical Chemistry, University of Dhaka, Bangladesh by measuring AUC for blood samples of the reference as well as the test animals in case of each of the pure drug as well as the drug-Mg complexes.

PHARMACOKINETIC MEASURES OF SYSTEMIC EXPOSURE[7]

Systemic exposure means comparable rate and extent of absorption. Exposure measures are defined relative to early, peak and total portions of the serum concentration-time profile, as follows.

For single-dose studies, the measurement of total exposure should be.

a) Area under the serum concentration-time curve from time zero to time t (AUC0-t), where t is the last time point with measureable concentration for individual formulation.

b) Area under the serum concentration-time curve from time zero to infinity (AUC0-α), where AUC0-α = AUC0-t +Ct/λz, Ct is the last measurable drug concentration and λz is the elimination rate constant.

Study design

The design of a pharmacokinetic study is dependent upon the drugs, dosage forms, animals used, test samples and study objectives. In this, study both the test and reference drug formulations were administered at the same dose of parent drug in the solution form by the oral route. The rats were fasted for 12 hours (overnight) prior to drug administration and kept fasting up to blood collection. The rats were divided into three groups having five rats in each group for each drug of study: control (rats without giving any drugs for each analysis) and group 1 for reference (single) drug and group 2 for test sample (drug-Mg complexes). The amount of dose

was given to the rat by considering average body weight 240-260 g. The dose of drug (both reference and test) was given in a specific time interval. After collection of blood, they were centrifuged and serums were collected and preserved in vials at 4oC. Then the concentrations (µg/mL) of drug and drug-Mg complexes in serum were analyzed by HPLC.

Dosage and drug administration

All the drugs were used in the solution form. Aspirin (both test and reference drugs) at a dose of 10 mg/kg body weight, paracetamol (both test and reference drugs) at a dose of 16 mg/kg body weight and naproxen (both test and reference drugs) at a dose of 16 mg/kg body weight were administered to the rats of specific group by oral route. Drug-Mg complexes were administered concomitantly at a dose of 10 mg/kg in the rats of specific group (group 2) by oral route.

Biological sampling schedule

For aspirin: After ingestion of drug, blood samples were collected from rat into a serum tube (red color clot activator, made in Becton, Dickinson and company, New Jersey, USA) at the time intervals of 0, 15, 30, 60, 120, 180 and 240 minute, respectively.

For paracetamol: After ingestion of drug, blood samples were collected from rat into a serum tube at the time intervals of 0, 30, 60, 120, 180 and 300 minute, respectively.

For naproxen: After ingestion of drug, blood samples were collected from rat in to a serum tube at the time intervals of 0, 30, 60, 120, 240, 360 and 480 minute, respectively.

Collection of Blood and Preparation of Evaluation Sample[8]

Blood was collected (i.v. only) from the treated rat and 0.7 mL of blood was transferred in to a tube. The anti-coagulated blood sample was transferred into a 1.5 mL eppendorf tube (VWR International, USA) and the tubes were centrifuged at 4000 rpm for at least 20 minute for the separation of serum. 250 µL serum was taken by micropipette into another eppendorf tube. The serum was diluted by 150 µL of methanol for denaturation of protein and 600 µL of ethanol for deproteinization. Then the samples were made ready for HPLC analysis or preserved immediately in refrigerator until analysis.

Statistical analysis

Statistical analysis was based on the guidelines for statistics and modified for the study of in vitro and in vivo trials.[9-11] The results were expressed as mean ± SD. Differences in mean values between experimental groups (in vitro and in vivo) were analyzed by one way analysis of variance (ANOVA), followed by Dunnett's multiple comparison tests where applicable. A 'p' value of 0.05 was taken as significant.

RESULTS

Since the drugs are readily absorbed from the gastrointestinal tract in the systemic circulation, after ingestion of drugs, blood was collected from rat into a serum tubes at the time intervals of 0, 15, 30, 60, 120, 180 and 240 minute, respectively for aspirin; 0, 30, 60, 120, 180 and 300 minute, respectively for paracetamol; and 0, 30, 60, 120, 240, 360 and 480 minute, respectively for naproxen. The drug levels in serum were analyzed by HPLC and the chromatograms are shown in the Figures 1-3.

In HPLC chromatograms the retention times of the aspirin, paracetamol and naproxen were obtained as 7.54, 4.68, 9.77 minute, respectively where as that of for Mg-complexes were found as 7.58, 4.67, 9.46 minute, respectively. Although the slight deviation of retention times are within the acceptable range but actually these occurred due to the complexation with magnesium.

Calculation of tmax, Cmax and AUC for aspirin.

The most important measurement of bioavailability is AUC. AUC was calculated directly from Cpvs time data. The simplest, most common approach, a numerical approximation method called the trapezoidal rule was employed. The AUC of each segment calculated as trapezoids. The area of each segment was also calculated by multiplying the average concentration by the segment width. Aspirin (both test and reference) was administered orally to rat at a dose of 10 mg/kg body weight. The drug concentration in serum and determined at 0, 15, 30, 60, 120, 180 and 240 minute, respectively. Inboth (test and reference aspirin) cases, the tmaxwas found at 15 min and Cmax for test and reference aspirin were found to be 11.24±0.08 and 11.39±0.06 µg/mL, respectively. Upon plotting the mean concentration vs time interval of both test and reference aspirin the following curve was obtained (Figure 4).The mean Cmax and AUC for aspirin were calculated by extrapolation of data based on curve and are shown in Table 1.

Calculation of tmax, Cmax and AUC for paracetamol

Paracetamol (both test and reference) was administered orally into rats at a dose of 16 mg/kg body weight. The drug concentration in serum was determined at 0, 30, 60, 120, 180, and 300 minute, respectively. In both (test and reference paracetamol) cases, the tmax was found at 60 min. and Cmax of test and reference paracetamol were 3.09±0.30 and4.39±0.34 µg/mL, respectively. Upon plotting the mean concentration vs time interval of both test and reference,paracetamol the following curve was obtained (Figure 5). Themean Cmax and AUC forparacetamol were calculated by extrapolation of data based on curve and is shown in Table 2.

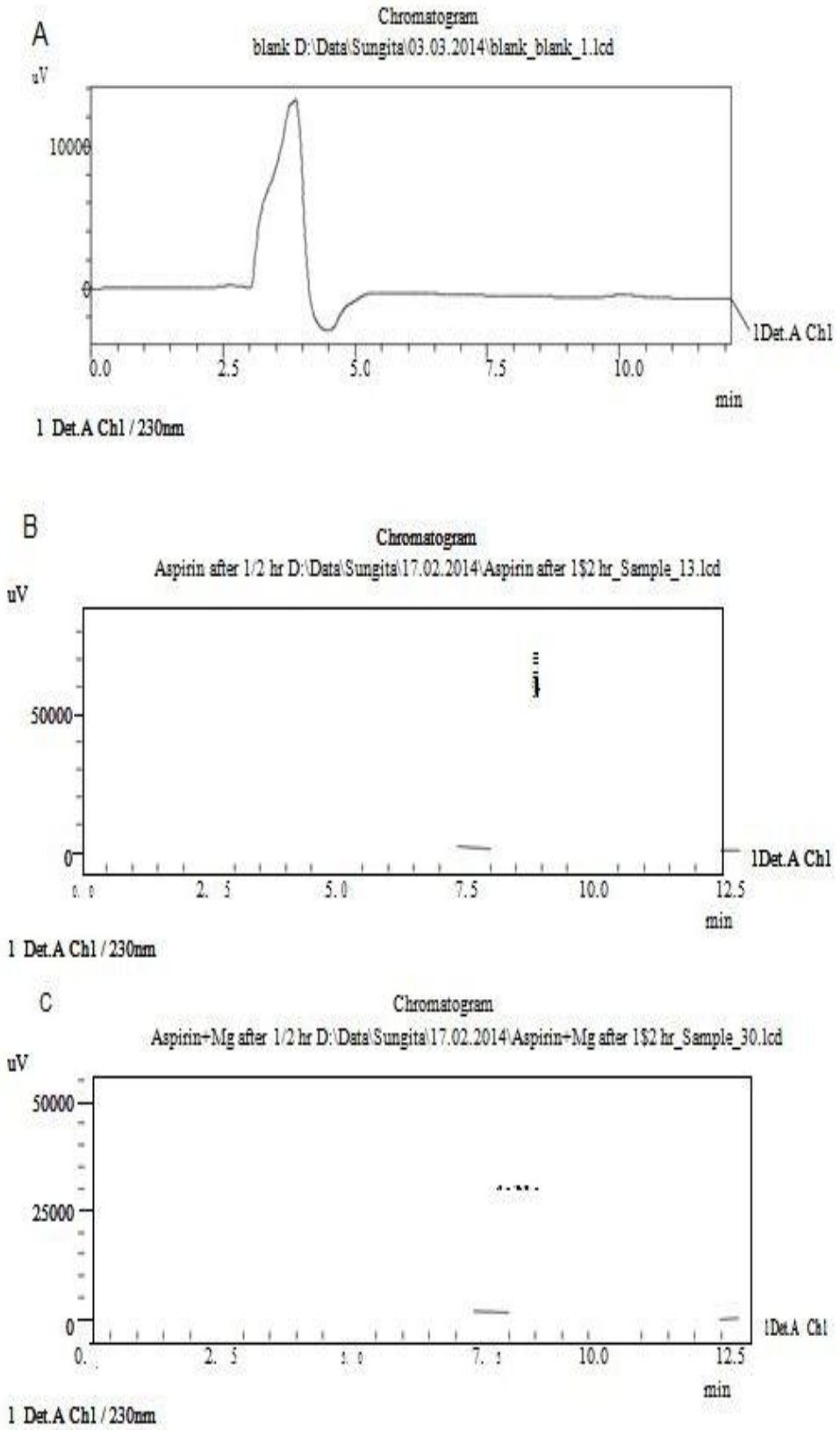

Figure 1: Chromatograms of serum (a), aspirin in serum (as reference) (b), aspirin with MgSO4 (c).

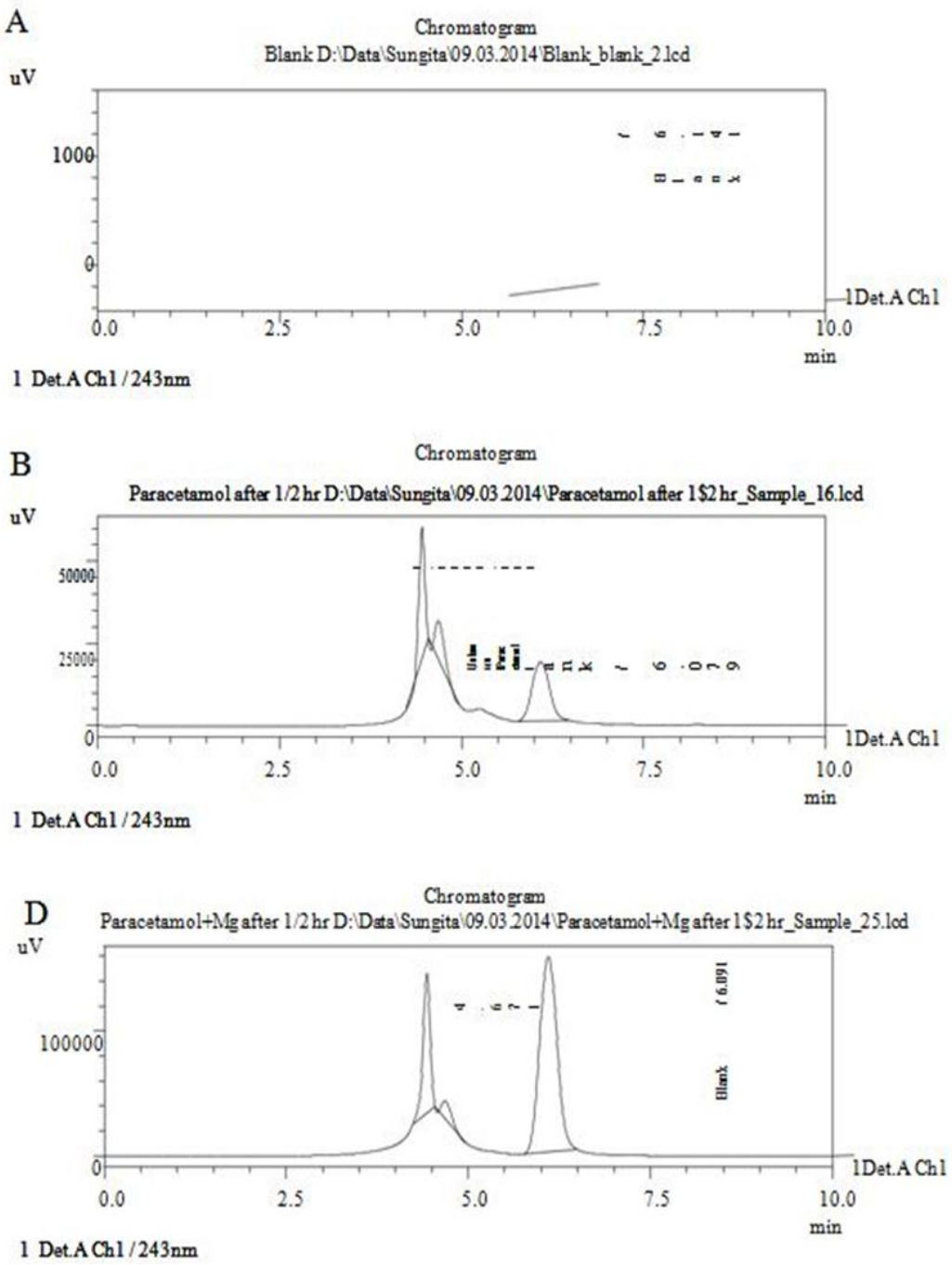

Figure 2: Chromatograms of serum (blank) (a), paracetamol in serum (as reference) (b), paracetamol with MgSO4 (c).

Calculation of tmax, Cmax and AUC of naproxen

Naproxen (both test and reference) was administered orally to rat at a dose of 16 mg/kg body weight. The drug concentration in serum was determined at 0, 30, 60, 120, 240, 360 and 480 minute, respectively. In both (test and reference) cases, the tmax of naproxen was found to be 120 min. and Cmax of naproxen was 25.22±0.24 and 26.49±0.35 µg/mL for test and reference, respectively. Upon plotting the mean concentration vs time interval of both test and reference naproxen the following curve is obtained (Figure 6). The mean Cmax and AUC for naproxen were calculated by extrapolation of data based on curve and is shown in Table No 3.

DISCUSSION

Aspirin is used in the treatment of mild to moderate pain, inflammation and fever. It is also used as an antiplatelet agent to prevent myocardial infarction, stroke and transient ischemic episodes. Aspirin is absorbed rapidly from the stomach and intestine by passive diffusion. Actually aspirin is transformed into salicylate in the stomach, in the intestinal mucosa, in the blood and mainly in the liver. Salicylate is the active metabolite responsible for most anti-inflammatory and analgesic effects but acetylsalicylate is the active moiety for the antiplatelet-aggregating effect.

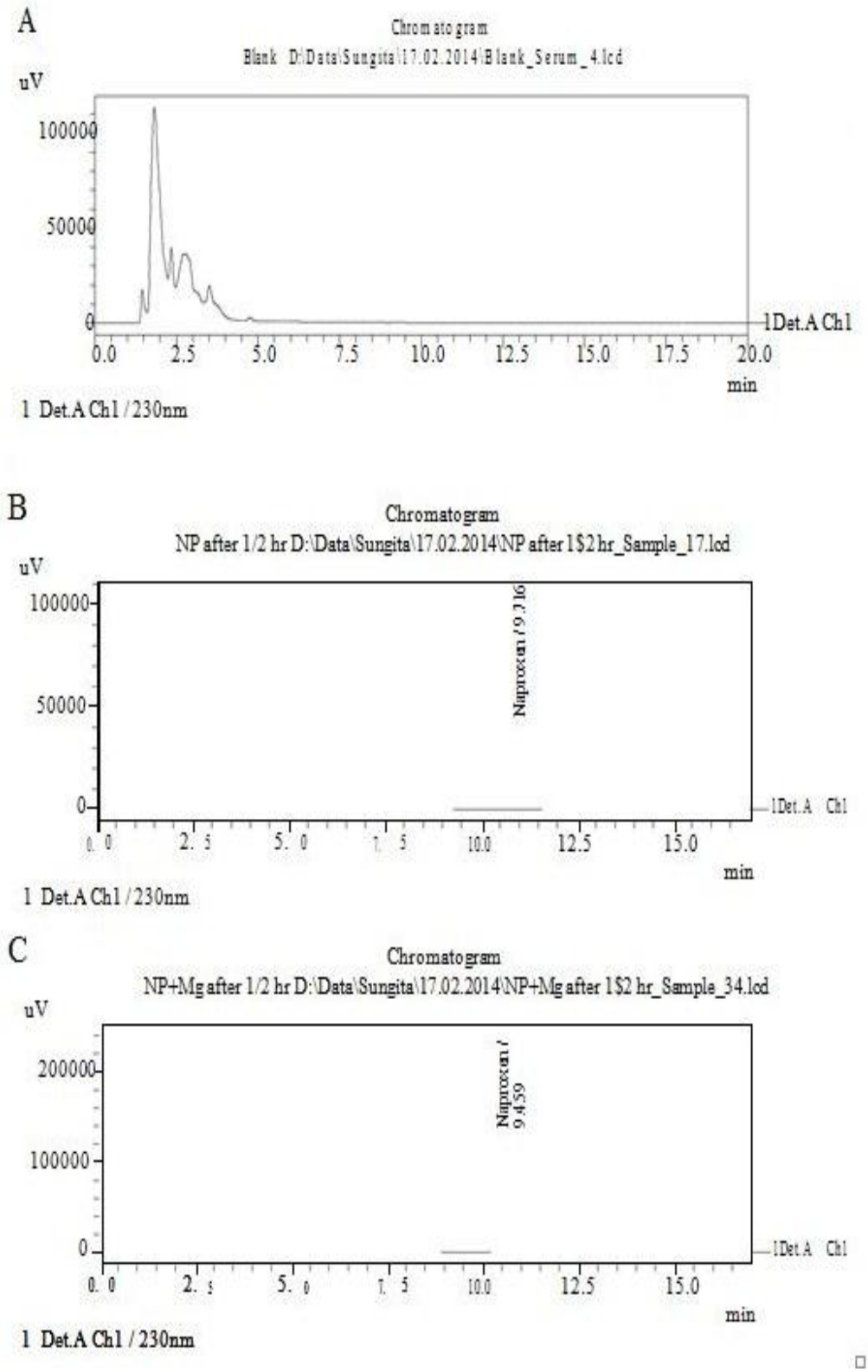

Figure 3: Chromatograms of serum (blank) (a), naproxen (as reference) (b), naproxen with MgSO4 (c).

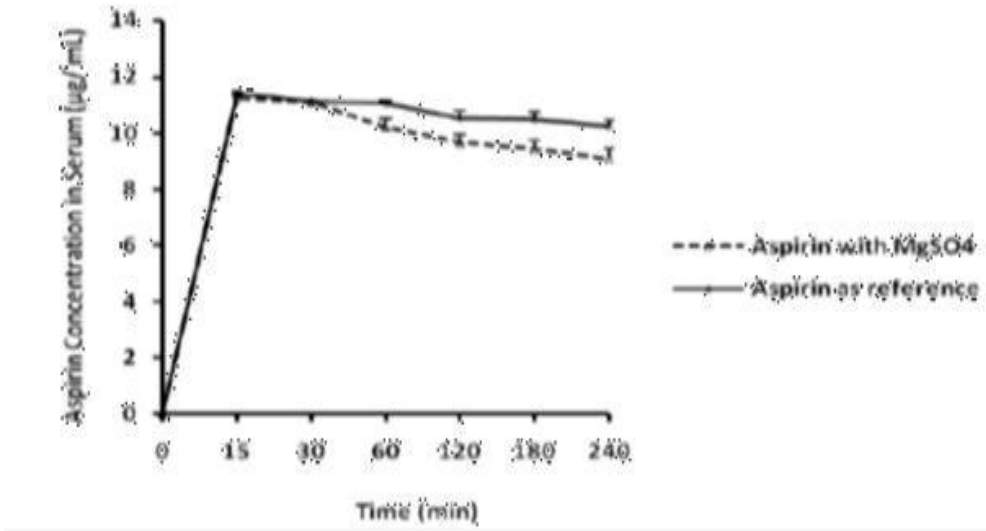

Figure 4: Mean concentration of aspirin vs time curve (both test and reference aspirin).

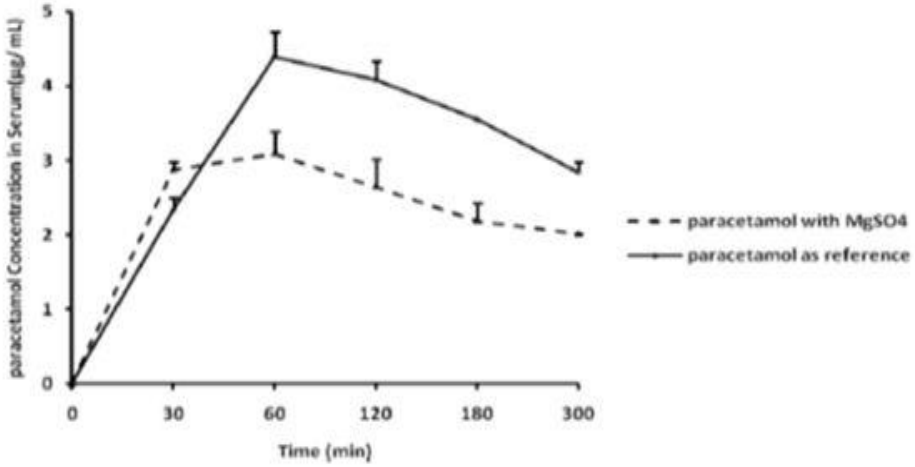

Figure 5: Mean concentration of paracetamolvs time curve (both test and reference).

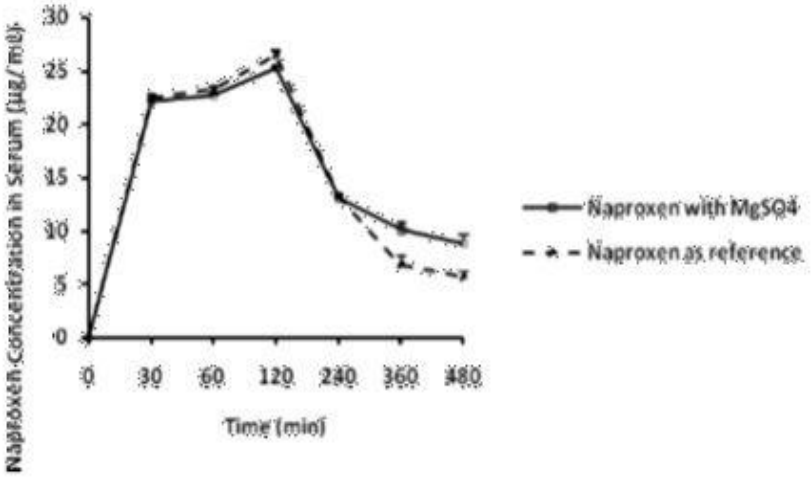

Figure 6: Mean concentration of naproxen vs time curve (both test and reference).

Table 1: Cmax, AUC(0-t) and AUC (0-α) of test and reference aspirin.

Group	Cmax ± SD	AUC (0-t) ± SD	AUC (0-α) ± SD
Aspirin as reference	11.39 ± 0.01	41.37 ± 0.05	45.05 ± 0.04
Aspirin with MgSO4	11.24 ± 0.05	38.24 ± 0.08	41.52 ± 0.08

Table 2: Cmax, AUC(0-t) and AUC (0-α) of test and reference paracetamol.

Group	Cmax ± SD	AUC(0-t) ± SD	AUC(0-α) ± SD
Paracetamol as reference	4.39 ± 0.01	16.71 ± 0.17	22.88 ± 0.47
Paracetamol with MgSO4	3.09 ± 0.01	11.72 ± 0.04	16.07 ± 0.03

Table 3: Cmax, AUC (0-t) and AUC (0-α) of test and reference naproxen.

Group	Cmax ± SD	AUC (0-t) ± SD	AUC (0-α) ± SD
Naproxen as reference	26.49 ± 0.01	114.18 ± 0.14	130.51 ± 0.13
Naproxen with MgSO4	25.22 ± 0.01	120.93 ± 0.01	146.35 ± 0.01

Salicylate distributes rapidly into the body fluid compartments. It binds to albumin in the plasma. With increasing total plasma salicylate concentrations, the unbound fraction increases. Salicylate, in turn, is mainly metabolized by the liver. As mentioned above, aspirin is rapidly biotransformed into the active metabolite, salicylate. Therefore, aspirin has a very short half-life.[6] The relative bioavailability of test aspirin was found 92% at 240 minutes, considering AUC0-t of test and reference aspirin and 92% after infinitive time, considering AUC0-α of test and reference aspirin. As it was known from the absorption profile of aspirin, half-life of this drug is 120 minutes, but in our study aspirin reached the steadystate at 240 minutes. It might be due to insufficient metabolism, increase in protein binding, comperatively high dose of aspirin for both test and reference drug; although from the above study it was found that concurrent administration of magnesiun with aspirin, it slightly increased elimination rate and lower the bioavailability than the reference aspirin.

In human beings, oral paracetamol shows excellent bioavailability. Peak plasma concentrations occur within 30–60 minutes and the t1/2 in plasma is about ~2 hour. Binding of the drug to plasma proteins is less in comparison to other NSAIDs.[12]

The relative bioavailability of test paracetamolwas found to be 70% at 300 minutes, considering AUC0-t of test and reference paracetamol and 70% after infinitive time (considering AUC0-α of test and reference paracetamol.

It was also found that concurrent administration of magnesiun with paracetamol markedly increased elimination rate and decreased the bioavailability than that of the reference paracetamol. It might be due to the increased plasma protein binding of paracetamol.

It has been already established that in human being naproxen is absorbed fully when administered orally. Maximum concentrations in plasma occur within 2–4 hour and are somewhat more rapid after the administration of naproxen sodium. Absorption is accelerated by the concurrent administration of sodium bicarbonate but delayed by magnesium oxide or aluminum hydroxide.[12] In our study in rats, the relative bioavailability of test naproxen was found to be 105% at 480 minutes, considering AUC0-t of test and reference naproxen and 112% after infinitive time, considering AUC0-α of test and reference naproxen.

It was observed that while magnesiun was concomitantly administered with naproxen, it decreased elimination rate and remained in systemic circulation for longer time which may be harmful for body or useful in tharapeutic quantities, often avoiding the need of freequent administration.

CONCLUSION

From the pharmacokinetic study of aspirin and paracetamol in rats, it was observed that concomitant administration of magnesium with aspirin, paracetamol and naproxen elimination rate was slighly increased and the bioavailability was lowered the single aspirin or paracetamol. But it was found that concurrent administration of magnesium salt with naproxen not only delayed the absorption rate of naproxen but also decreased its elimination rate and remained in systemic circulation for longer time than the single administered naproxen. The retention of naproxen for longer time in systemic circulation may be harmful for body from tharapeutic consideration. It might be inferred that the combination of NSAID drugs with magnesium might be discouraging or encouraging which can be ascertained only after a longer course of study.

ETHICAL APPROVAL

All authors hereby declared that all experiments were performed in accordance with the ethical standards laid down in the Declaration of Helsinki 1964.

ACKNOWLEDGEMENT

None.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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