Synthesis, characterization and biochemical study of novel ester prodrugs containing aspirin and ibuprofen

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Abstract
Aspirin and ibuprofen are aryl acids of nonsteroidal anti inflammatory drugs with good analgesic. For reducing the gastrointestinal toxicity associated with presence free carboxylic acid functional group in aspirin and ibuprofen, therefore the present work is aimed to synthesize a novel ester prodrugs of these two drugs. These two novel prodrugs were prepared by the reaction of aspirin or ibuprofen acid chloride with 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose, then deprotection of ester prodrug of aspirin was also accomplished using 70% acetic acid. The purity of the synthesized compounds was established by (TLC), column chromatography, while their structures were confirmed by (FT-IR, ^{1}HNMR, ^{13}CNMR). Then 36 rabbits has been obtained and divided to six groups to show the bioavailability of the prepared prodrugs at some biochemical parameters and compared with the parent drugs and control group. Statistical analysis revealed significant increases in the levels of creatine kinase, alkaline phosphatase, aspartate aminotransferase, alanine amino transferase, glutathione, malondialdehyde, creatinine, uric acid, concentration of Cu, and significant decreases in the levels of choline esteras, total protein, Zn concentration, while the results showed no significant increases in the activity of lactate dehydrogenase, acid phosphatase and no significant decreases in the levels of albumin, globulin, and iron concentration for the prepared prodrugs compared with the control group. Finally, to ensure the release of parent drugs(aspirin or ibuprofen), hydrolytic studies of the ester prodrugs were obtained at different pH (2, 4, 10 and 12) at 25^{o}C, the results showed that the hydrolysis at basic pH were faster than acidic pH, so it means that the most hydrolysis will obtain at intestine not at stomach.
Keywords: Prodrug, Aspirin, Ibuprofen, NSAIDs

تحضير ودراسة بايوكيميائية وتشخيصية لادوية مصاحبة استريه تحتوي الاسبرين والايبوبروفين

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الخلاصة
يعتبر الاسبرين والايبوبروفين من الاحماض الاريلية للأدوية المضادة للالتهابات الغير ستيرويدية ومسكن جيد للألم ، ولتقليل السمية الناتجة من وجود مجموعة الكاربوكسيل الفعالة في الاسبرين والايبوبروفين، تم في هذا البحث تحضير مركبات استرية جديدة كادوية مصاحبة للدوائين اعلاه، حيث حضرت من الادوية المصاحبة للأسبرين والايبوبروفين من تفاعلهما ككلوريدات حوامض مع 1,2:5,6-ثنائي-O-ايزوبروبلدين-α-D-كلوكوفيورانوز، ومن ثم ازالة مجموعة الحماية لمشتق الاسبرين المحضر بواسطة التحلل بحامض الخليك 70%. وتم تنقية المركبات المحضرة بكروموتوغرافيا العمود واستخدام ال TLC، حيث تم التأكد من صحة التحضير بتقنيات (FT-IR, ^{1}HNMR, ^{13}CNMR). بعد ذلك استخدم 36 ارنب تم تقسيمهم الى ست مجاميع لمعرفة مدى الفعالية البايولوجية للادوية المصاحبة المحضرة على بعض المتغيرات الكيموحيوية ومقارنة نتائجها مع استخدام الادوية الاساسية ومجموعة السيطرة. نتائج التحليل الاحصائي بينت وجود زيادة معنوية في مستويات الكرياتين كاينيز، الفوسفاتيز القاعدي، الاسبارتيت امينوترانسفيريز، الالانين امينوترانسفيريز، الكلوتاثايون، المالون ثنائي الالديهايد،الكرياتنين واحامض اليوريك، وفي تركيز النحاس، وكذلك انخفاض معنوي في مستويات انزيم الكولين استريز والبروتين الكلي، وكذلك في تركيز الزنك، بينما بينت النتائج انخفاض غير معنوية في مستويات الالبومين، الكلوبيولين وتركيز الحديد للأدوية المصاحبة المحضرة مقارنة بمجموعة السيطرة.

واخيرا، تم التاكد من تحررالادوية (الاسبرين والايبوبروفين) من الادوية المصاحبة الاسترية بواسطة تحليلها بمحالين مختلفة الحامضية (pH 2, 4, 10, 12) والنتائج بينت ان التحلل في الوسط القاعدي اسرع من التحلل في الوسط الحامضي وهذا يعني ان معظم التحلل سوف يحصل بالامعاء وليس في المعدة.

الكلمات المفتاحية : الدواء المصاحب، الاسبرين، الايبوبروفين، الادوية المضادة للالتهابات الغير ستيرويدية

Introduction

Generally, a drug is characterized by its biological and physicochemical properties. Some of the used drugs have undesirable properties that result in an inefficient delivery and unwanted side effects. The physicochemical, biological and organoleptic properties of these drugs should be improved in order to increase their usefulness and their utilization in clinical practice[1,2]. Classical NSAIDs, inhibit COX-1 activity and block the biosynthesis of prostaglandins that protect the gastric mucosa, they also cause local irritation by the free carboxylic acid contained in their structures. Both factors are the source for the gastrointestinal complications resulted from the use of NSAIDs [3] . It is widely believed that eliminating the gastrointestinal side effects and reducing the toxicity associated with the NSAIDs use can be achieved via chemical modifications of the main functional groups contained in the NSAID structure [4] . Ideally, the design of an appropriate prodrug structure should be considered at the early stages of preclinical development, bearing in mind that prodrugs might alter the tissue distribution, efficacy and the toxicity of the parent drug. Some of the most common functional groups that are amenable to prodrug design include carboxylic, hydroxyl, amine, phosphate/phosphonate and carbonyl groups. Prodrugs typically produced via the modification of these groups include esters, carbonates, carbamates, amides, phosphates and oximes [5]. For these reasons, carboxyl and hydroxyl groups in drugs are often targeted for derivatization toward the development of prodrugs[6]. Therefore, utilizing the prodrug approach might improve the physicochemical properties of a drug and hence increase its bioavailability and therapeutic efficiency [6,7]. The prodrug have been extensively and successfully used as a chemical tool for modification of the physicochemical, pharmacokinetic as well as pharmacodynamic characteristics of commonly used drugs and new drugs[8]. In most cases, prodrugs contain a promoiety (linker) that is removed by enzymatic or chemical reactions, while other prodrugs release their active drugs after molecular modification, such as an oxidation or reduction reactions [9]. Therefore our aim is designing a novel esters prodrugs of the aspirin and ibuprofen by using , 1,2:5,6–di–*O*–isopropylidene–α–D–glucofuranose as a carrier alcohol which containe a free OH group at position 3, and we hope that the combination of these two drugs with carbohydrate molecule by regioselective esterification reactions may lowering toxicity compared with parent drugs, and also increased lipophilicity.

Methods

Chemistry part

The synthetic designing plane of this work is illustrated in figure (1) below

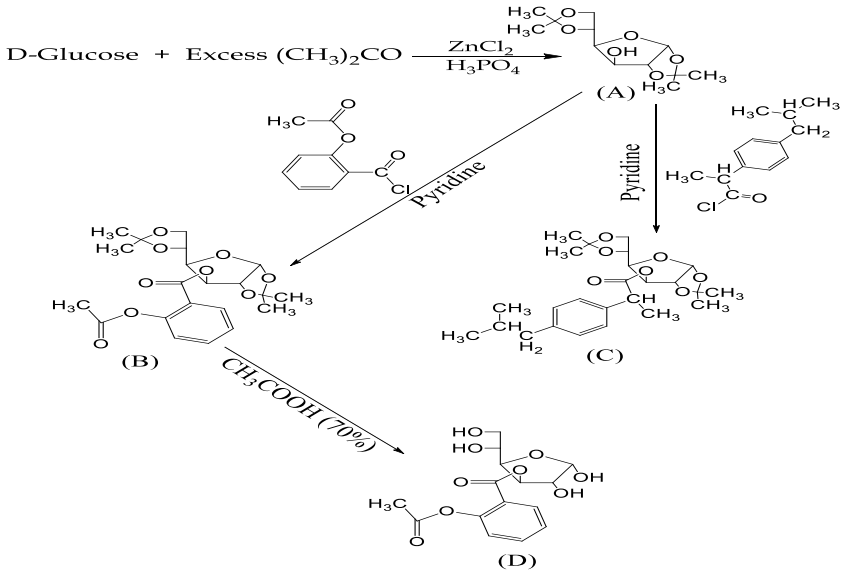

Fig. (1): Synthesis of the prodrugs

1-protection of OH groups

Synthesis of 1, 2 : 5, 6 – di – O – isopropylidene – α – D –glucofuranose (A)[10]

Compound(A) was prepared according to the literature procedure by stirring a mixture of (100gm, 0.55 mole) anhydrous glucose, (750 mL) acetone, (71.6 gm) anhydrous $ZnCl_2$ and (2.9 mL) of H_3PO_4 for 30 hour at room temperature.

Analysis

M.P. (100-105)^oC, R_f (0.41) (chloroform : Diethyl ether) (4:1), IR (KBr Disc), (Ʊ cm^{-1}), 3431(OH), 2987-2873(C-H)aliphatic,C-O-C asymmetrical and symmetrical (1377,1058).

2-Synthesis of Aspirin and Ibuprofen acid chloride [11]

These two drug acid chlorides were synthesized according to the known procedure by refluxing gently of the parent drug with excess of thionyl chloride($SOCl_2$), then the prepared acid chlorides were used directly in the next step.

3-Synthesis of 3-*O*-[(2-acetoxy)benzoyl]-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (B)[12]

(2.63 gm-10.131 mmol) of compound (A) was dissolved in dry pyridine at (-12^oC), 2-acetoxy benzoyl chloride (aspirin acid chloride) was added gradually (dropwise) to the mixture. The mixture was stirred for 24 hour, then water (100 mL) was added, the mixture was extracted three times (3*100 mL) chloroform. The chloroform layer was separated and washed with 10% HCl and dried with anhydrous $MgSO_4$, then the final product was collected by distilling the chloroform through rotary evaporator and purified with column chromatography.

Analysis: (Yield, 2.8621gm, 66.87%), R_f (0.80)(chloroform:Diethyl ether) (4:1), IR (Film) (Ʊmax cm^{-1}), 2987-2941 (C-H) aliphatic , 1731-1756 (-O-CO-Drug , -O-CO-CH3)Ester, 1608 (C=C)aromatic, (C-O-C) asymmetrical and symmetrical (1253, 1078), 750(C-H) aromatic out of the plane),. ^{1}H-NMR (DMSO) δ(ppm),: 1.2-1.6 (4s,12H,4CH_3 isoprop.), 2.1-2.3(s,3H,CH_3CO Ester), 3.6-4.9 (m, H6a, H6b, H-5, H-4, H-3, H-2) , 5.2(d,1H, H-1, anomeric) , 7.3-7.9(m,4H, aromatic). ^{13}C-NMR, δ(ppm),: 21.29 (1C, CH_3, CO aliphatic), 25.2-28.8(4C,isoprop.) , 60.3-85.1 (6C , C6 , C5 ,C3 ,C4 ,C2), 106.06(C1, anomeric),109-113 (2C, isoprop.), 124.41-150.65, (6C ,aromatic), 164-169.59 (2C,2C=O,Ester).

4-Synthesis of 3-*O*[2'-(4''-isobutyl phenyl)propionyl]-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (C)[12]

(2.37gm-9.126 mmol) of compound (A) was dissolved dry pyridine at (-12°C), 2-(4-isobutyl phenyl)propionyl chloride(ibuprofen acid chloride) was added gradually (drop wise) to the mixture. The mixture was stirred for 24 hours, then water (100 mL) was added, the mixture was extracted three times (3*100 mL) chloroform. The chloroform layer was separated and washed with 10% HCl and dried with anhydrous $MgSO_4$, then the final product was collected by distilling the chloroform through rotary evaporator and purified with column chromatography (silica gel as stationary phase and methanol as a mobile phase) and follow up the fractions by TLC.

Analysis

(yield, 2.97 gm ,72.76%), R_f (0.96) (chloroform:Diethyl ether)(4:1), IR (Film) (U_{max} cm^{-1}), 2950(C-H) aliphatic,1745 (C=O)Ester, 1475-1510(C=C), C-O-C asymmetrical and symmetrical (1245,1020) succession, 840 (C-H) aromatic out of the plane , ^{1}H-NMR (DMSO) δ(ppm): 0.8-1.0 (d, 6H, $2CH_3$ aliphatic), 1.2-1.5(4s ,12H, $4CH_3$, isopr), 1.8(3H,CH3 aliphatic) ,2.2 (1H,CH aliphatic) , 2.4(s,2H, CH_2, aliphatic), ,3.6 (m,1H, CHCO) ,3.9-4.6 (m, 6H, H6a , H6b , H5, H4, H3, H2, isoprop), 5.0-5.1 (m,1H,anomeric) , 7.0-7.3 (2b ,4H , aromatic) ,^{13}C-NMR, δ(ppm):19.3-23.2 (3C aliphatic), 26-30.1(4C,isopro), 31.1(1C aliphatic) ,45.4 (1C aliphatic), 67.2-85.8 (6C ,C6 ,C5 ,C3 ,C4 ,C2), 105(1C ,C1 anomeric) ,106-112(2C isoprop), 124-130 (4C,2b, aromatic), 137-140 (2C, C1,C4 aromatic), 175(1C C=O Ester).

5-deprotection of the OH groups

Synthesis of 3-*O*-[(2-acetoxy)benzoyl]-α-D-glucofuranose (D)[13]

A mixture of compound (B)(2 gm, 4.730 mmole) and 70% acetic acid (25 mL) was stirred for 24 hour at room temperature, the mixture was neutralized using sodium bicarbonate, then diluted with distilled water (25 mL), extracted 3 times with chloroform, the organic layer separated and dried over anhydrous $MgSO_4$, the solution was filtered and evaporated under reduced pressure, then purified by column chromatography to afford the final product.

Analysis

(yield, 0.6028 gm ,37.19%) R_f (0.25) (chloroform:Diethyl ether) (4:1) or R_f(0.72) (absolute ethanol) IR (Film) (Umax cm-1), 3480 (OH) ,2995 (C-H) aromatic , 2930 (C-H)aliphatic ,1750 (C=O)Ester ,1610 (C=C) aromatic, 740 (C-H) aromatic out of the plane. ^{1}H-NMR (DMSO) δ(ppm):1.9 (s, 4H,4OH, D-Glucose) ,2.0-2.1 (s,3H,CH_3CO, Ester) , 3.3-5.0(m, 6H, H6a, H6b, H-5, H-4, H-3, H-2) ,5.9 (d, 1H, anomeric), 7.2-7.9 (m, 4H, aromatic), ^{13}C-NMR, δ(ppm): 22.4(1C,CH_3CO-Ester), 63.1 -85 (C6 ,C5 ,C4, C3, C2, Glucose), 105 (1C,C1 Glucose, anomeric), 124-135 (5C, aromatic),151(1C, C6, aromatic), 170 -175 (2C, C=O Ester).

Biochemistry part

In this study six rabbits groups had been obtained, every group contain six rabbits (weight≤2Kg), before withdraw blood samples, the rabbits leaves hungry for 24 hours, four from these groups were gave oral dose (25 mg\Kg) from the prepared compounds and parent drugs (dissolved by DMSO), and one of the divided group obtained as a solvent effective group, then the last group used as a control group. The table below show the names of groups

Table (1):- The names of groups

Group (A)	Gave aspirin (6 rabbits)
Group (B)	Gave Ibuprofen (6 rabbits)
Group (C)	Gave compound (C) (6 rabbits)

Group (D)	Gave compound (D) (6 rabbits)
Group (E)	Control group(6 rabbits)
Group (F)	Gave DMSO (6 rabbits)

By disposable syringe (5 mL) of blood withdraw, centrifuged, and the serum was separated from blood by micropipette to keep the sample under (-20^{o}C) for the biochemical tests. Determination of cholinesterase activity(Biolabo Kit)[14] has been obtained by the hydrolysis of Butyrylthiocholine to Butyrate and Thiocholine. Thiocholine reduces yellow hexacyanoferrate (III) to colorless hexacyanoferrate (II). The decrease in absorbance at 410 nm was directly proportional to the cholinesterase activity in the sample. Creatine kinase-MB(CK-MB)(Biolabo Kit) [14], modified reagent contains a polyclonal antibody (specific to the CK-M monomer) which so completely inhibits CK-MM activity and one half of CK-MB activity. Only the activity of the non-inhibited B monomer subunit, representing half of the CK-MB activity, is measured. The method assumes that CK-BB activity in the specimen is essentially zero. The increase in absorbance due to the conversion of $NADP^{+}$ into NADPH, measured at 340 nm, was proportional to the CK-MB activity in the specimen. The activity of peroxidase (Biolabo Kit)[15], was routinely measured by the spectrophotometric determination of generated hydrogen peroxide by the reaction with a chromogenic hydrogen donor in the presence of peroxidase using the 4-aminoantipyrine/phenol system according to Allain et al The estimation of alkaline phosphatase(ALP) activity(Syrbio Kit) [14] obtained by using a colorimetric method. free phenol liberated by hydrolysis of the substrate reacts then with 4-amino-antipyrine in the presence of alkaline potassium ferrocyanide to form a red-color complex which absorbance measured at 510 nm is directly proportional to the ALP activity in the specimen**.** The colorimetric method has been obtained to determine the aspartate aminotransferase(AST) and alanine amino transferase (ALT) by using Tonhanzy and White method (Biolabo Kit) [14], while Reitman and Frankel were resolved the method of measuring the activity of enzymes at serum. Aspartate aminotransferase was measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenyl-hydrazine while, alanine aminotransferase was measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenyl-hydrazine. The glutathione(GSH) assay is carried out by the modified method which first described by Tietze [16]. The general thiol reagent, 5-5'-dithiobis[2-nitrobenzoic acid] (DTNB, Ellman's Reagent) reacts with GSH to form the 412 nm chromophore, 5-thionitrobenzoic acid (TNB) and GS-TNB. The GS-TNB is subsequently reduced by glutathione reductase and b-nicotinamide adenine dinucleotide phosphate (NADPH), releasing a second TNB molecule and recycling the GSH; thus amplifying the response. Any oxidized GSH (GSSG) initially present in the reaction mixture or formed from the mixed disulfide reaction of GSH with GS-TNB is rapidly reduced to GSH. The assay of malondialdehyde was based on the reaction of malondialdehyde (MDA) with thiobarbituric acid (TBA), forming a MDA-TBA2 adduct that absorbs strongly at 532 nm.[17] The determination of magnesium level has been obtained by Ginder, Heth and Khayam method according to the literature [14]. The principles of the method was to procuration complex of Mg with 1-hydroxy-4-methyl-2-phenylazo]-2-naphtol-4-sulphoric acid and measure the

absorption at 500-510nm. After dissociation of iron-transferrin bound in acid medium, ascorbic acid reduced Fe^{+3} iron into Fe^{+2} iron. Fe^{+2} iron then form a colored complex with 3-(2-pyridyl)-5,6-difuryl-1,2,4-triazine-disulfonate (ferine). The absorbance thus measured at 580-620 nm was directly proportional to the amount of iron in the specimen [18]. Thiourea was added in the reagent to prevent the copper interference. Determination of Copper and Zinc is carred out as stated elsewhere[19], a standards of copper and zinc must be prepared. The metals were dissolved in deionized water to prepare a concentrated stock solution. The stock solution was diluted to prepare the analytical standards, then the measuring of the copper and zinc levels at blood serum obtained by atomic absorption spectrophotometer technique (AAS). Finally, the release of parent drugs (Aspirin, Ibuprofen) were approved by the hydrolysis of the ester prodrugs [D],[C] respectively at different pH [2, 4, 10, 12] and at (25^{o}C) by using buffer solutions.

Results and discussion

The strategy of this work involving protection of the 1,2 and 5,6 hydroxyl groups of the D-glucose followed by direct regioselective esterification of the hydroxyl group at position 3, therefore, our first task was to prepare 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose(A) by treatment of D-glucose with excess anhydrous acetone and $ZnCl_2$ according to the literature procedure. The characterization of the carrier alcohol compound (A) was accomplished by measuring physical and IR-spectral data, the melting point of this compound is (105-109)^{o}C, and it was identical to that reported in the literature[10]. The IR spectrum of this compound showed the presence of the hydroxyl group band at (3431)cm^{-1} being broad due to intermolecular hydrogen bonding. Its IR spectrum also showed a band at (2987, 2873)cm^{-1} for C-H aliphatic. Conversion of acid groups of ibuprofen and aspirin to their corresponding acid chloride is necessary step to increase the reactivity of the carbonyl group in acid part and also necessary for the better condensation esterification reaction which was resulted in good yield of the parent ester prodrug, for this reaction thionyl chloride was used to prepare 2-(4-isobutyl phenyl)propionyl chloride and (2-acetoxy)benzoyl chloride, then the reactions of these acid chlorides with carrier acetonated D-glucose(A) through selective esterification of the hydroxyl group at position 3, the progress of the esterification reaction can be followed readily by examine melting points, IR , ^{1}HNMR and ^{13}CNMR spectral data. It is worthwhile to mention her, that the melting points of these two esters(semi solids) are lowered than that of the acid drug and alcohol, this probably due to the disappearance of the hydrogen bonding in ester prodrug. The disappearance of the broad absorption band in the range (3431)cm^{-1}due to hydrogen bonding (O-H) stretching together with the appearance of a stretching bands at (1731, 1756)cm^{-1}, assigned to the (–O-CO-CH_3 and -O-CO-Drug) respectively, which may considered a good evidence to improve the successful esterification to produced these novel two ester prodrugs (B) and (C), figure(2 and 3).

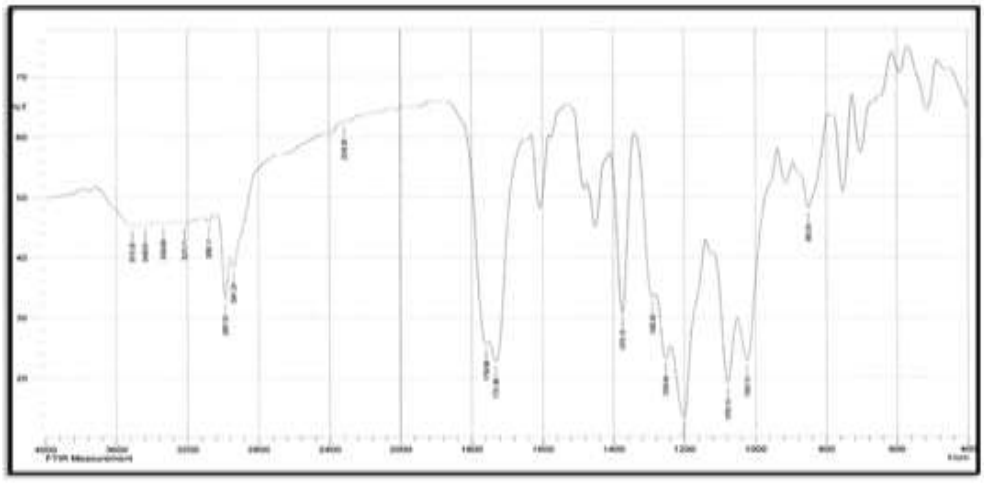

Fig.(2):- IR spectrum for derivative(B)

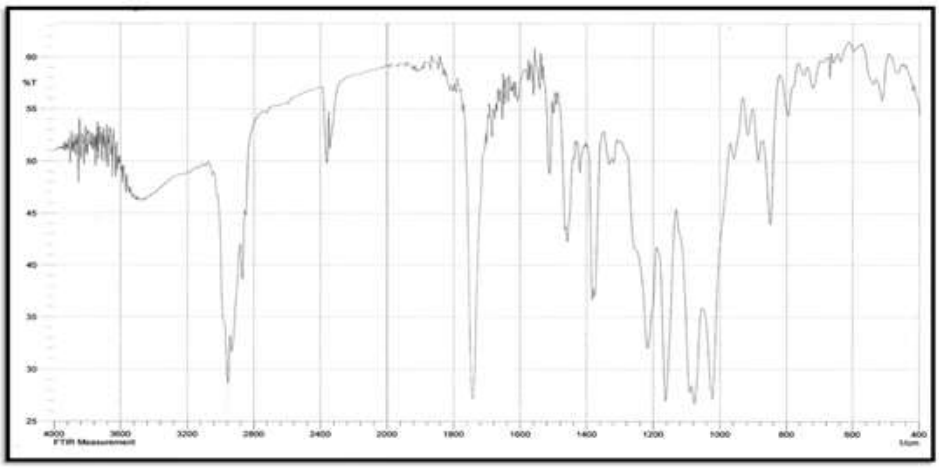

Fig.(3):- IR spectrum for derivative(C)

The structures of these two prodrugs were also established by studying the ^{1}HNMR data showed that aspirin and ibuprofen acid moieties were exclusively grafted onto C-3 position of carrier molecule. The ^{1}HNMR of (B), figure(4) showed 2.1-2-3(S, 3H, CH_3CO Ester) acetate of aspirin, and 7.3-7.9(m, 4H, aromatic)for benzene ring of aspirin, and disappearance of OH proton at C-3 of glucose which indicate that the link has performed at position 3. The ^{1}HNMR for (C), figure(5) showed absorbance at 0.8-1.0(d, 6H, $2CH_3$ aliphatic), 2.2(1H, CH aliphatic) and 2.4 (s,2H,CH_2, aliphatic) for propionyl group of ibuprofen, 1.8(3H,CH_3 aliphatic) for isobutyl group of ibuprofen and 7.3-7.9(m, 4H,aromatic) for benzene ring of ibuprofen.

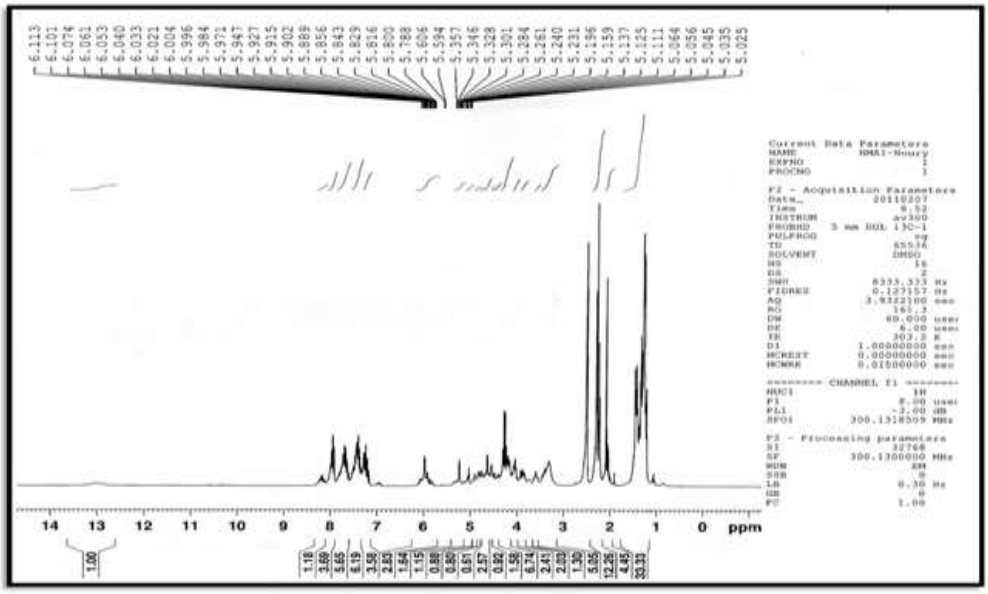

Fig.(4):- ^{1}HNMR spectrum for derivative(B)

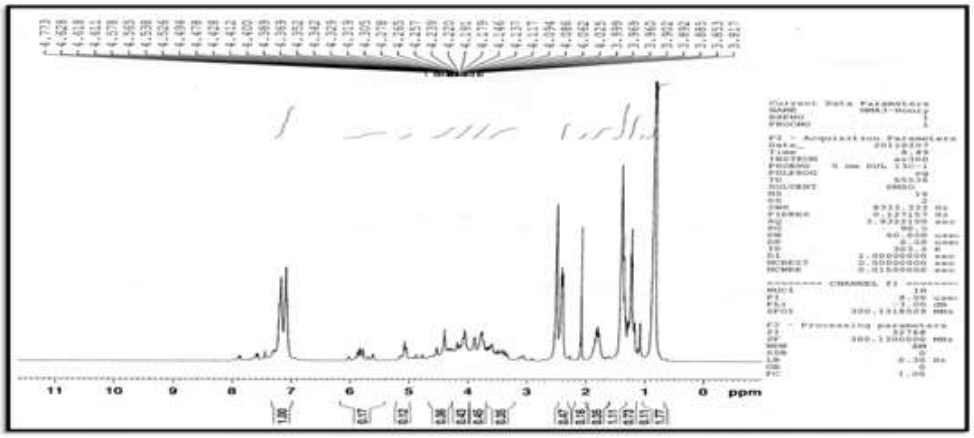

Fig.(5):- ^{1}HNMR spectrum for derivative(C)

Furthermore, ^{13}CNMR of (B), figure(6) showed 21.29 (1C, CH_3CO aliphatic), 124.41-150.65(6C ,aromatic), 164-169.59 (2C,2C=O Ester) of aspirin, and ^{13}CNMR of (C), figure(7), showed 137-140 (2C, C1, C4 aromatic) benzene ring of ibuprofen and 175 (1C, C=O Ester) of ibuprofen

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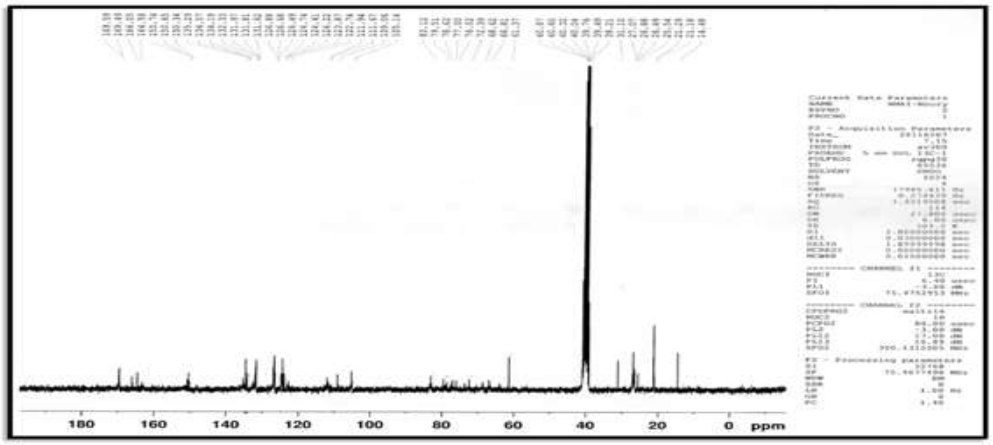

Fig.(6):- ^{13}CNMR spectrum for derivative(B)

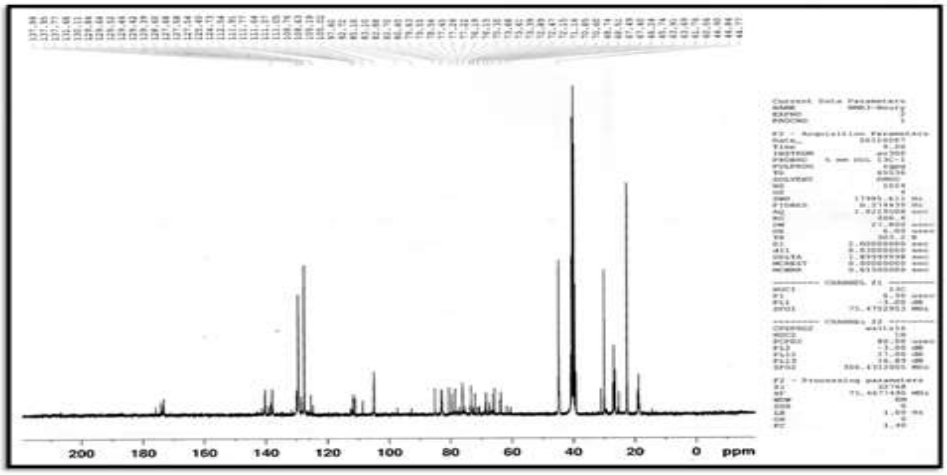

Fig.(7):- ^{13}CNMR spectrum for derivative(C)

Acid catalyzed hydrolysis of aspirin prodrug using 70% acetic acid leading to deprotection the two isopropylidene groups in position 1,2 and 5,6. The occurring of ketalic hydrolysis was indicated using (FT-IR, ^{1}HNMR and ^{13}CNMR) spectral data, the FT-IR spectrum, figure(8) give the following stretching and bending vibration (cm^{-1}), the appearance of the broad band in the (3480)cm^{-1} due to hydrogen bonding(O-H) stretching together with the appearance of stretching band around (1750)cm^{-1} assigned to the carbonyl(C=O) of ester group which was a good evidence for the occurrence of the ketal hydrolysis and not ester hydrolysis. The ^{1}HNMR of (D), figure(9), showed disappearance of two isopropylidine, 2-2.1 (S, 3H, CH_3CO Ester) of aspirin, 7.2-7.9(m, 4H, aromatic) benzene ring of aspirin mean that the hydrolysis did not hydrolyze the ester bond between aspirin and glucose. ^{13}CNMR of (D) , figure(10), showed disappearance the absorbance of two isopropylidene groups, 22.4(1C, CH_3CO-Ester), 170 -175(2C C=O Ester), and 151(1C, C6, aromatic) of aspirin. The (C) derivative leave without hydrolysis of isopropylidene group to know the effect of this derivative which have more lipophylisity than the other.

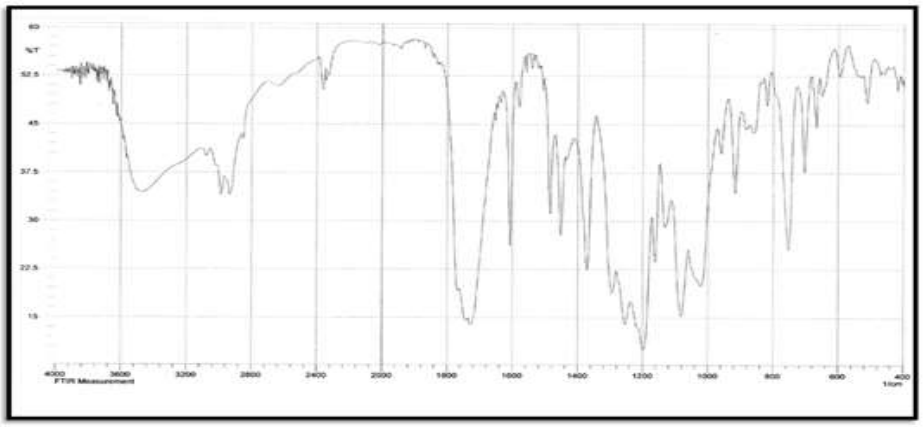

Fig.(8):- IR spectrum for derivative(D)

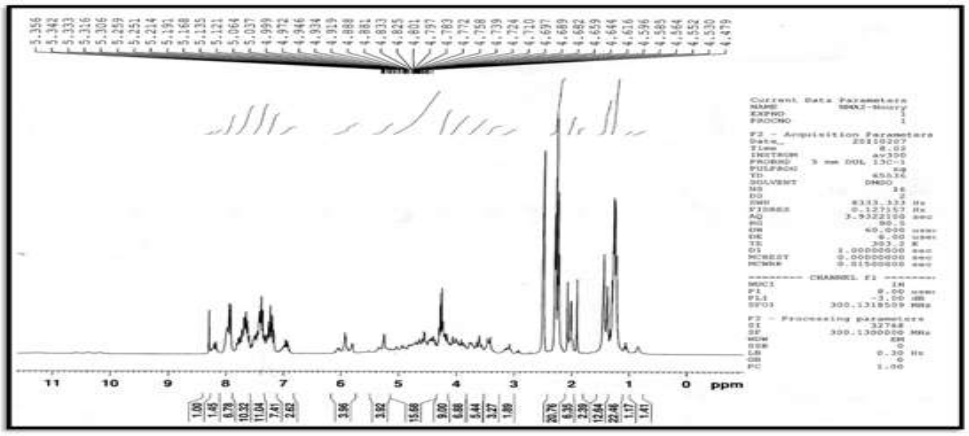

Fig.(9):- ^{1}HNMR spectrum for derivative(D)

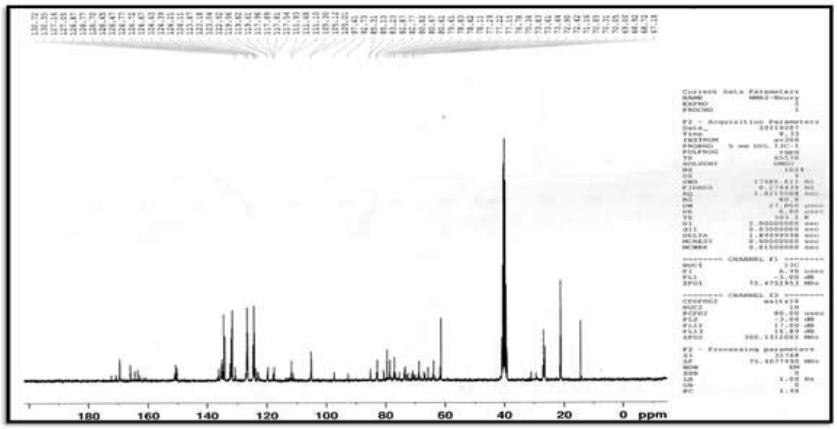

Fig.(10):- ^{13}CNMR spectrum for derivative(D)

Biochemistry part

After the preparation of derivatives C and D, and gave the written doses as shown at the used animals and samples collection part**,** the effect of these derivatives on the measured parameters were shown at the tables (2,3,4 and 5). Table (2) show the effect of C and D derivatives on choline esterase(ChE) creatine kinaseCK-MB and peroxidase.

Table (2):- Groups, mean ± SD, P-value of Choline esterase and Creatine Kinase CK(MB)

Parameters	Groups	Mean ± SD	p-Value
Choline esterase (ChE) U/L	E	4163.13±662.32	0.009
	D	2708.40±348.29	
	E	4163.13±662.32	0.0001
	C	2748.90±464.21	
	A	3140.43±295.54	0.128
	D	2708.40±348.29	
	B	2964.57±351.77	0.508
	C	2748.90±464.21	
Creatine kinase CK-MB U/L	E	93.559±24.125	0.004
	D	157.785±21.590	
	E	93.559±24.125	0.03
	C	146.995±29.314	
	A	112.115±8.885	0.013
	D	157.785±21.590	
	B	147.130±16.176	0.993
	C	146.995±29.314	
peroxidase U/L	E	15.333±2.565	0.0001
	D	29.500±3.555	
	E	15.333±2.565	0.001
	C	31.150±4.932	
	A	22.733±6.773	0.151
	D	29.500±3.555	
	B	26.433±7.722	0.274
	C	31.150±4.932	

The results of ChE at table (2) showed a significant decrease at the activity of enzyme by the effect of C and D compared with the control group E, and the values were 2748.90±464.21 , 2708.40±348.29 and 4163.13±662.32 U/L respectively, while there were a non significant decrease at C and D compared with the parent drug A and B, thus, the results suggest that the prepared derivatives possess inhibitory effect little more than the parent drugs, that may be due to the nature of enzyme in the analysis of ester linkage [20], including aspirin, ibuprofen and the prepared derivatives are all esters, therefore, it is expected that these compounds have been associated with the active site of enzyme causing inhibitory effect rather than the substrate.[21] furthermore, the results at table (2) showed significant increase on creatine kinase CK-MB activity caused by the effect of C and D groups compared with the control group (E), the CK-MB values were 146.995±29.314, 157.785±21.590 and 93.559±24.125 U/L respectively. While, the results showed no significant effect between the prepared derivatives and the parent drugs. aspirin and other NSAIDs causes lowering metabolic pathway at liver by inhibit the mitochondrial lactate dehydrogenase so lead to lowering the energy (ATP) that causing activation of creatine kinase to compensates of the shortfall in energy by the (ATP) configuration from creatine phosphate.[20]The results at table(2) also,

showed significant increase on peroxidase by the effect of C and D compared with the control group, the peroxidase levels were 31.150±4.932, 29.500±3.555 and 15.333±2.565 U/L respectively, and no significant effect between the prepared derivatives and the parent drugs. These results are consistent with that obtained by Hasegawa et.al[22], that aspirin leads to the reduction of oxidative stress and increase in the activeness of enzyme.

Table (3):- Groups, mean ± SD, P-value of ALP, AST and ALT

Parameters	Groups	Mean ± SD	p-Value
ALP U/L	E	90.515±12.571	0.005
	D	121.933±13.061	
	E	90.515±12.571	0.012
	C	129.158±17.052	
	A	100.636±13.467	0.028
	D	121.933±13.061	
	B	107.383±15.496	0.058
	C	129.158±17.052	
AST U/L	E	54.764±10.799	0.002
	D	75.366±5.032	
	E	54.764±10.799	0.007
	C	77.560±6.681	
	A	68.456±6.031	0.157
	D	75.366±5.032	
	B	72.731±5.533	0.211
	C	77.560±6.681	
ALT U/L	E	15.322±3.062	0.018
	D	26.863±7.470	
	E	15.322±3.062	0.068
	C	22.973±6.052	
	A	30.521±6.365	0.337
	D	26.863±7.470	
	B	31.387±3.005	0.018
	C	22.973±6.052	

Table(3) showed significant increase in the activity levels of ALP and AST by the effect of C and D derivatives compared with the control group, the values of ALP were 129.158±17.052, 121.933±13.061 and 90.515±12.571 U/L, as well as the values of AST were 77.560±6.681, 75.366±5.032 and 54.764±10.799 U/L respectively. While there were no significant effect at ALP and AST between the prepared derivatives and the parent drugs. These results are consistent with that obtained by Udegbunam et.al[23], they found increasing in the level of effectiveness of the liver enzymes due to the treatment with ibuprofen. As well as, the reason for this increasing may be due to the damage of the cell membrane and causing the exit of fluid out of the wall which lead to the liberation of liver enzymes in the blood vessels and causing a

high percentage of it. Also, the results at table(3) showed a significant increase in the level of ALT by the effect of the prepared derivative D 26.863 ±7.470 compared with the control group 15.322±3.062, non significant increase by the effect of C 22.973±6.052 compared with the control group 15.322±3.062, non significant decrease by the effect of D compared to the parent drug A, and significant decrease (inhibitory effect) by the effect of C 22.973±6.052 compared with the parent drug B 31.387±3.005.

Table (4):- Groups, mean ± SD, P-value of GSH and MDA

Parameters	Groups	Mean ± SD	p-Value
GSH µmol/L	E	4.158±0.321	0.012
	D	4.957±0.501	
	E	4.158±0.321	0.043
	C	4.771±0.366	
	A	4.710±0.320	0.288
	D	4.957±0.501	
	B	4.897±0.448	0.703
	C	4.771±0.366	
MDA µmol/L	E	7.986±1.778	0.0001
	D	14.240±2.861	
	E	7.986±1.778	0.0001
	C	14.409±1.712	
	A	11.507±1.841	0.046
	D	14.240±2.861	
	B	12.744±2.275	0.04
	C	14.409±1.712	

Table (4) showed significant increase in the level of GSH effected by C and D compared with the control group, and the values were 4.771±0.366, 4.957±0.501 and 4.158±0.321 µmol/L respectively, and no significant effect between the prepared derivatives and the parent drugs. The reason of the increase may be due to the decrease of free radicals generated and thus an increase in the concentration of glutathione, as noted by Hasegawa et.al[22], that the aspirin leads to the reduction of oxidative stress which leads to increase in the activeness of enzyme, and the results are consistent with that obtained by Sundaram et.al[24] that there are a reverse correlation between glutathione concentration and oxidative stress. The results at table(4) also showed significant increase in the level of MDA effected by C and D compared with the control group, and the values were 14.409±1.712, 14.240±2.861 and 7.986±1.778 µmol/L respectively, and same results between the prepared derivatives C and D 14.409±1.712 and 14.240±2.861 µmol/L compared with the parent drugs A and B 11.507±1.841 and 12.744±2.275 µmol/L. The reason of this increase may be due to play these esters(the prepared derivatives) as a primer at the peroxidation process, so these esters compounds are oxidized in acidic media of stomach because they antioxidant group and convert to the negative ion[25], and associated with H^+ of the fatty acids and hydrolyzed it and give malondialdehyde at final of the process. Wagner et.al[26] noted that the hydrolysis of unsaturated fatty acids at cell membrane by the self motivation chain reactions of free radical and the dehydrogenation of the unsaturated fatty acids by a primer to continues the chain

hydrolysis(propagation). Michael[27] also noted that the lipids peroxidation at the cell membrane due to influence on the permeability of the cell membrane, and this is shown by the effect of these compounds to increase the activity of the liver enzyme, whereas the increase of these enzymes were evidence for the effect of the cell wall that caused by the highly lipid peroxidation process, another meaning, these compounds causing double effect, decreasing lipids peroxidation and oxidative stress, so that meaning it have the ability to treatment the increasing of free radicals in inflammatory conditions, but at the same time it due to increasing of lipid peroxidation at this dose.

Table (5):- Groups, mean ± SD, P-value of Mg, iron, Cu and Zn

Parameters	Groups	Mean ± SD	p-Value
Mg mmol/L	E	0.635±0.049	0.426
	D	0.686±0.179	
	E	0.635±0.049	0.004
	C	0.953±0.147	
	A	0.543±0.113	0.151
	D	0.686±0.179	
	B	0.557±0.088	0.009
	C	0.953±0.147	
Iron mmol/L	E	35.398±10.500	0.055
	D	46.084±13.092	
	E	35.398±10.500	0.216
	C	42.522±8.960	
	A	49.919±18.517	0.627
	D	46.084±13.092	
	B	45.683±7.288	0.431
	C	42.522±8.960	
Cu µmol/L	E	21.009±0.692	0.005
	D	25.367±1.656	
	E	21.009±0.692	0.004
	C	25.003±2.008	
	A	23.964±2.043	0.352
	D	25.367±1.656	
	B	24.740±1.449	0.829
	C	25.003±2.008	
Zn µmol/L	E	19.418±0.569	0.0001
	D	12.025±1.306	
	E	19.418±0.569	0.0001
	C	11.654±2.194	
	A	13.810±0.904	0.034
	D	12.025±1.306	
	B	16.112±1.565	0.021
	C	11.654±2.194	

Finally, table(5) showed significant increase in the level of Mg effected by C when compared it with the control group, and the values were 0.953±0.147 and 0.635±0.049 mmol/L respectively, similarly the same effect has been obtained by C when compared with the parent drug B, and the values were 0.953±0.147 and 0.557±0.088 mmol/L respectively, while no significant increase in the levels of Mg effected by D when compared it with the control group and with the parent drug A. The rise in the level of magnesium may be due to the reduction of glomerular filtration[28]. The results of iron at table(5) showed significant increase effected by D when compared it with the control group, and the values were 46.084±13.092 and 35.398±10.500 respectively, while no significant increase in the level of iron effected by C when compared it with the control group, and no significant decrease effected by the two derivatives C and D when compared them with the parent drugs A and B respectively. Furthermore, the results at table(5) showed significant increase in the level of Cu effected by C and D when compared it with the control group, and the values were 25.003±2.008, 25.367±1.656 and 21.009±0.692 µmol/L respectively, and no significant increase effected by the two derivatives C and D when compared them with the parent drugs A and B respectively. The liver is the main controlling the metabolism of copper because copper found in the liver cells associated with metallothionein, so the rise in the level of copper may due to the effect of the prepared derivatives on the liver cells and causing defect in the function of liver[29], and another cause due to rise the level of copper noted by Burtis &Ashwood[30] , some ions compete with copper like zinc to link with the –SH located at the active site of metallothionein, so the increase of copper concentration offset by decreases in the concentration of zinc, and that it the final results at table(5), the results showed significant decrease in the level of Zn effected by C and D when compared it with the control group, and the values were 11.654±2.194, 12.025±1.306 and 19.418±0.569 µmol/L respectively, and significant decrease effected by the two derivatives C and D when compared them with the parent drugs A and B, and the values were 13.810±0.904, 12.025±1.306, 11.654±2.194 and 16.112±1.565 µmol/L respectively. The prepared derivatives may be stimulate the zinc to link with the –SH group at the active site of metallothionein instead of copper [30]. The ratio of drug releasing has been calculated by using specrtophotometric methods, the releasing of drug follow up by UV at λ_{max}=275 nm for aspirin and λ_{max}=261nm for ibuprofen, figure(2) show the percentage hydrolysis at different pH.

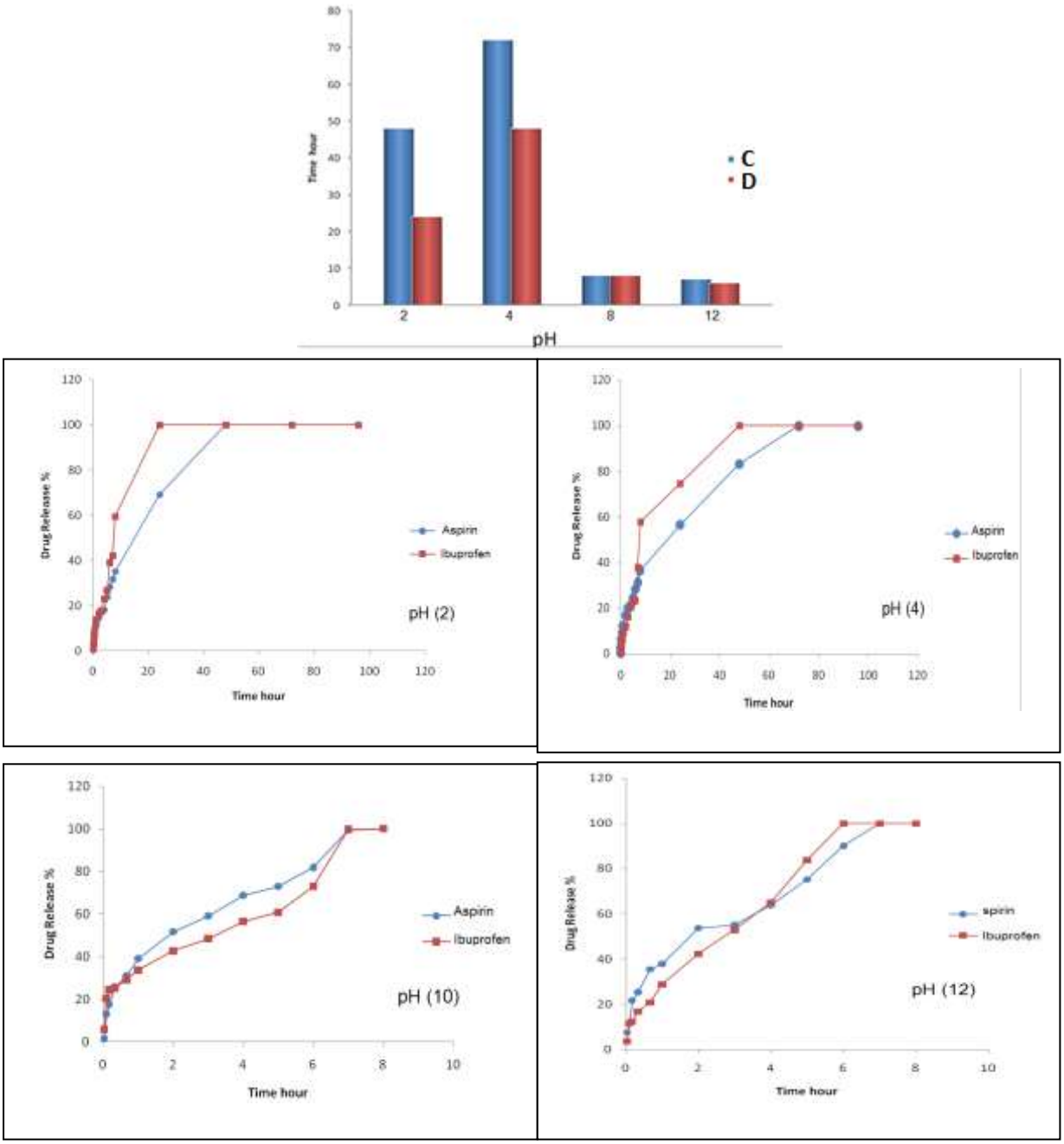

Fig.(2):- Releasing of prodrugs derivatives at different pH

The results showed that the hydrolysis at basic pH were faster than acidic pH, so it mean that the most hydrolysis will obtain at intestine not at stomach.

Conclusions

Inhibitory effect in (C and D) groups compared with the control group, and more strongly inhibition than the parent drugs. A significant increase in the activity of CK-MB, peroxidase, ALP, AST and ALT at (C and D) groups compared with the control group. A significant increase in the levels of GSH, MDA, iron and Cu in (C and D) groups compared with the control group. A significant decrease in the level of Zn at (C and D) groups compared with the control group. The most hydrolysis of the

prepared derivatives will obtain at intestine not at stomach which that mean the prepared derivatives reduce the side effects of aspirin and ibuprofen and their toxicity at stomach.

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