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Role of Ezetimibe in Combination with Statins(Simvastatin and Atorvastatin) in Controlling Dyslipidemia

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Abstract

Cardiovascular risk is independently increased by plasma lipids abnormalities (low- density and high density lipoprotein -cholesterol and triglycerides). Most patients have more than one lipid abnormality. Combination therapy with lipid-modifying agents could offer an important therapeutic option for improving the overall lipid profile. Combinations have demonstrated to provide additive efficacy and significant reductions in coronary events . This study was designed to evaluate the effect of ezetimibe, when used in combination with other hypolipidaemic agents (statins) on lipid profile as well as on liver function ,renal function, oxidative stress, and platelets function when given to dyslipidaemic patients . Forty four patients (24 males and 20 females) with age ranged between 40-70 years (54 ±14.6) with dyslipidaemia on statins therapy for at least 6 month were involved in this clinical trials. They were randomized into two groups treated with either a combination of 20 mg/day simvastatin or a combination of 20mg/day atorvastatin and 10mg/day of ezetimibe.The study also included 22 apparently healthy subjects with age ranged (40-70years) and sex(11males and 11 females) matching that of the patients group. Serum lipid profile (total cholesterol -TC, triglycerides -TG, low density lipoprotein-cholesterol –LDL-C, very low density lipoprotein-cholesterol-VLDL-C, and high density lipoprotein-cholesterol –HDL-C), oxidative stress marker (Malondialdehyde-MDA), liver functions indices (Alanin aminotransferase -ALT,Aspartate aminotransferase- AST, total bilirubin), renal function parameters (urea, creatinine, and microalbuminuria) and platelets function test (bleeding time)were evaluated before and after 4 and 6 weeks of starting ezetimibe treatment . Treatment with ezetimibe plus simvastatin or atrovostatin resulted in significant lowering in TC, TG, LDL-C levels with elevation in HDL-C also the LDL/HDL ratio lowered significantly (by 38.16%). This effect was associated with significant changes in liver function , and oxidative stress without changes in platelets function nor in renal function. The results presented in this study indicated that ezetimibe can be used in clinical practice for the treatment of dyslipidaemia, when combined with other hypolipidaemic agents like simvastatin and atorvastatin to improve the therapeutic profile with ameliorating some of their adverse effects.

Keywords : Ezetimibe , Statins , Dyslipidemia

الخلاصة

ان خطر أمراض الأوعية القلبية يمكن إن يزداد بصورة غير معتمدة عند الاختلال في الدهون الثلاثية والدهون البروتينية عالية الكثافة وواطئة الكثافة. وجد ان معظم المرضى لهم أكثر من خلل واحد في الدهون. ان العلاج المركب من المواد المعدلة للدهون يوفر فائدة علاجية مهمة لتحسين كل مستويات الشحوم في الدم . و العلاج المركب يمكن ان يكون له فعالية إضافية مسبباً هبوطاً معنوياً في التأثيرات على الشرايين التاجية. أجريت هذه الدراسة وصممت لتقييم فعالية الايزتايمب مع مواد خافضة للدهون مثل الستاتينات على معايير الكيمياء الحياتية والمتمثلة بمستويات ايض الدهون و فرط الأكسدة ووظائف الكبد والكلى ، إضافة إلى تأثيره على وظائف الصفيحات الدموية (زمن النزف) ومقارنة هذه التأثيرات مع أدوية تقليدية خافضة للدهون (سمفاستاتين و اتورفستاتين) كنظام مختلط مع الازيتياميب عند مرضى الشحام .اشتملت هذه الدراسة على (44) مريضا (24 ذكور، 20 اناث) بعمر يتراوح بين (40-70) سنة وبمعدل 54± 14.6 مريضاً بداء الشحام مستمرين على العلاج بادوية الستاتين. تم تقسيم المرضى عشوائياً الى مجموعتين كالأتي:
المجموعة الأولى: هي مجموعة المرضى الذين استخدموا نظام مختلط بين السمفاستاتين والايزيتايميب 20+10 ملغم يومياً والمجموعة الثانية على نظام مختلط بين الاتورفاستاتين والايزيتايميب 20+10 ملغم يومياً. استمرت فترة المتابعة ستة أسابيع متتالية. كذلك ضمن الدراسة مجموعة مقارنة من الأصحاء بأعمار مقاربة وبنفس توزيع الجنس لمجاميع المرضى بالشحام وبعدد (22). تم قياس مستويات الشحوم في الدم (TC,TG,HDL,LDL) ومعايير فرط الاكسدة المالوندالديهايد ووظائف الكبد (AST,ALT T.Bil.) ووظائف الكلى (S.Urea,creatinine,MAU) ووظائف الصفيحات الدموية (Bleeding Time) قبل اعطاء العلاج وبعد مرور 4 اسابيع من اعطاء الازيتاميب. اظهرت تحاليل البيانات فروقا معنوية واضحة للازيتاميب مع السمفاستاتين او الاتروفاستاتين على مستوى شحوم الدم حيث لوحظ حصول انخفاض معنوي في تراكيز (TC,TG&LDL) وارتفاع مستوى HDL وانخفاض ملحوظ في نسبة

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LDL/HDL ترافق مع تغير معنوي في وظائف الكبد .ALT,AST,T.Bil . كذلك ظهر تحسن معنوي في معايير فرط الاكسدة متمثلة بانخفاض مستوى MD A و لم يتم ملاحظة أي تأثيرات ظاهرة او سلبية ذات دليل معنوي لوظائف الكلى والكبد والصفيحات الدموية.في ضوء النتائج التي افرزتها هذه الدراسة يمكن استنتاج مايلي :ان الازيتاميب يمكن ان يستخدم كعلاج اضافي بكفاءة لعلاج مرض الشحام وعندما يؤخذ مع ادوية خافضة للكوليسترول كالسمفاستاتين والاتروفاستاتين يمكن ان يحسن الفائدة العلاجية ومنع التأثيرات الجانبية المتوقعة.

Introduction

Dyslipidemia can be the result of a genetic predisposition, secondary causes or a combination of both.[1] The major lipid components of serum, Cholesterol and triglycerides can produce three forms of dyslipidemia: Hypercholesterolemia, hypertriglyceridemia and a combination of both. In each case, the dyslipidemia is the result of an elevation in either the number or composition of specific lipoproteins , which is an important determinant for selecting the appropriate drug therapy.[2,3].The NCEP guidelines for diagnosis of dyslipidemia, however, are based on clinical cut point that indicates relative risk for coronary disease. Including the general recommendation that total cholesterol and HDL levels to be measured every five years beginning at age 20 in persons who do have a family history of coronary heart or other atherosclerotic disease.[4] . LDL is considered as the primary atherogenic lipoprotein, and the smaller the size of the LDL particle, the more it is able to penetrate into subendothelial tissue, thereby contributes to the development of atherosclerosis.[5] For people with CHD, several large trials have demonstrated that aggressive lipid lowering is beneficial in people with CHD with considering the following points:-

A target LDL. Cholesterol level below 70-80 mg/dl is recommended for people who have CHD and have multiple major risk factors (e.g patients with diabetes or who smoke). Patients who experience myocardial infraction (MI) should be started on the cholesterol lowering medication while in the hospital and are advised to make life style changes , regardless of their LDL-cholesterol level.A target LDL-cholesterol level less than 100mg/dl is recommended for people who have CHD but do not have many additional risk factors.Life style changes as well as medications may be recommended when LDL levels are greater than 100mg/dlWhilest for people without a history of CHD also appear to benefit from lipid lowering therapy although the treatments are not as aggressive as in patients with CHD [6] . Five major classes of drugs are available now for the treatment of dyslipidemia , each with different effects on the various lipids and lipoprotein profile (Table-1) [7,8]. Statins are the most potent drugs available now for reducing LDL-C, they bring about moderately lower triglyceride level and modestly increase HDL-C levels [9] .

Table (1) : Major classes of drug used in treating dyslipidemia [2]

Drug class	LDL-cholesterol	HDL-cholesterol	Triglycerid
Statins	↓ **18%-55%**	↑ **5%-15%**	↓ **7%-30%**
Bile acid sequestrates	↓ **15%-30%**	↑ **3%-5%**	**No change or increase**
Niacin	**5%-25%**	**15%-35%**	↓ **20%-50%**
Fabric acid	↓ **5%-20%** **May be increased in patients with high triglyceride level**	↑ **10%-20%**	↓ **20%-50%**
Cholesterol absorption inhibitors	↓ **17%-19%**	↑ **1%-4%**	↓ **0%-6%**

Statins are considered the first line treatment of hypercholesterolemia in patient who have failed to adequately respond to dietary therapy.[10,11] Currently available products include Simvastatin, Atorvostatin, Lovastatin , Pravastatin, Fluvastatin Rosuvastatin [12]. oral agent that competitively inhibits HMG-CoA reductase, the catalytic enzyme in the conversion of HMG-CoA to mevalonic acid in the rate limiting step of cholesterol biosynthesis . [13]Recently, a potential mechanism for poor response to statin therapy was described by Patel et al(2001), [14] where the poor responders had a low basal rate of cholesterol synthesis that may be secondary to a genetically determined increase in cholesterol absorption possibly mediated by a polipoprotein E4 or by polymorphism in the HMG-CoA reductase gene.[15,16] Generally statins are contra-indicated in active liver disease (or persistently abnormal liver function tests) and in pregnancy (adequate contraception required during treatment and for 1 month afterwards) and breast-feeding. [17]The side effects of statins are reversible myositis which is a rare but a significant side-effect of the statins. Simvastatin and atorvastatin also cause headache; altered liver-function tests (rarely, hepatitis) and gastro-intestinal effects including abdominal pain, flatulence, diarrhea, nausea and vomiting. Rash and hypersensitivity reactions (including angioedema and anaphylaxis) have been reported rarely. [17] The new class of lipid modifying agents, cholesterol absorption inhibitors, acts to lower LDL-C concentrations by almost 20% regardless of concurrent therapy, and have a modest effect on HDL-C and triglycerides[18].Ezetimibe (Zetia, Merck/ Schering-plough pharmaceuticals) is the first agent approved in this class, might be a good option for patients who do not tolerate or respond to statin therapy .However, this product is contraindicated in patients with active liver disease. Ezetimibe acts through selective inhibition of intestinal cholesterol absorption . [19] Experimental studies suggest that ezetimibe prevents dietary and biliary cholesterol uptake that transport across the intestinal wall. [20,21] Ezetimibe -glucuronide, the primary metabolite, is transported from the liver back to the intestine in bile, and is a more potent inhibitor of cholesterol absorption than ezetimibe itself .[22]Relatively high level of fecal ezetimibe (69% of the administered dose) suggests limited absorption and possible hydrolysis of the glucuronide metabolite .[23] The dose of ezetimibe 10mg once daily.[17] The present study was designed to evaluate the possible effects of adding 10 mg daily dose of Ezetimbe (for 4 and 6 weeks) to hyperlipidemic patients ongoing with statins therapy (simvastatin 20 mg or atorvastatin 20 mg/day) on different components of lipoproteins in plasma, some biochemical markers for assessing liver , kidney and platelets function ,as well as , serum MDA levels.

Materials and Methods

This study was carried out in Al-Basrah General Hospital by selecting 44 patients (24 males and 20 females) with age ranged between 40-70 years (54 ± 14.6) presented with hyperlipidaemia (serum total cholesterol >200mg/dL) for more than 6 months on statins ,not having any CVD, from December 2006 to march 2007 . Twenty two apparently healthy subjects with comparable age and weight were also involved in this study as a control .Fasting blood specimens were utilized for assessing lipid profile (total serum cholesterol [24], triglyceride[25], and high density lipoprotein-cholesterol (26), low density lipoprotein-cholesterol [27] Liver function tests(Alanine minotransferase-ALT (Aspartate aminotransferase -AST [28] total bilirubin [29] , renal function tests (urea[30], creatinine[31] and microalbominurea -MAU [32] , platelets function tests (Bleeding time IVY method) [33] and oxidative stress (serum malondialdehyde –MDA[34]. Subjects were randomized into five groups:

Group 1: which include 22 apparently healthy subjects (11 male, 11 female) that not received any therapy during the study.

Group 2: which include 11 (6 male and 5 female) dyslipidaemic patients treated with Simvastatin 20 mg orally given as single daily dose at bed time for 6 weeks interval.

Group 3: which include 11 (6 male and 5 female) dyslipidaemic patients treated with Atorvastatin 20 mg orally given as single daily dose at bed time for 6 weeks interval.

Group 4: which include 11 (6 male and 5 female) dyslipidaemic patients treated with Simvastatin 20 mg + Ezetimibe 10 mg orally taken at bed time for 6 weeks interval.

Group 5: which include 11 (6male and 5 female) dyslipidaemic patients treated with Atrovastatin 20 mg + ezetimibe 10 mg orally taken at bed time for 6 weeks interval .

All values were expressed as means ± standard error of mean. Data were analyzed by in dependent T-test to assess the difference between two groups. P value less than 0.05 was considered significant).[35]

Results

Both simvastatin and atorvastatin treated groups (figure-1), showed no significant change in serum total cholesterol after 4 weeks of ezetimibe therapy ,but after 6 weeks of the addition of ezetimibe to simvastatin, there was a significant lowering in serum total cholesterol level as compared with both baseline . However , simvastatin treated group showed significant ($p<0.05$) reduction in serum TG levels after 4 weeks from the addition of ezetimibe (-28.6%)as compared with normal values , after 6 weeks from addition of ezetimibe it produced (-30.69%) reduction as compared with normal values (figure-2).While, 4 weeks of ezetimibe addition to atorvastatin produced a significant lowering ($p<0.05$) in serum TG level as compared to both normal and baseline values (-31.93%and -29.36%, respectively). After 6 weeks serum TG levels were lowered by(-19.6%,-17.4%) as compared with normal and baseline values respectively.However, the hyperlipidemic group treated with simvastatin exert non significantly elevated levels of serum HDL-C after 4 weeks from the addition of ezetimibe (figure-3) .

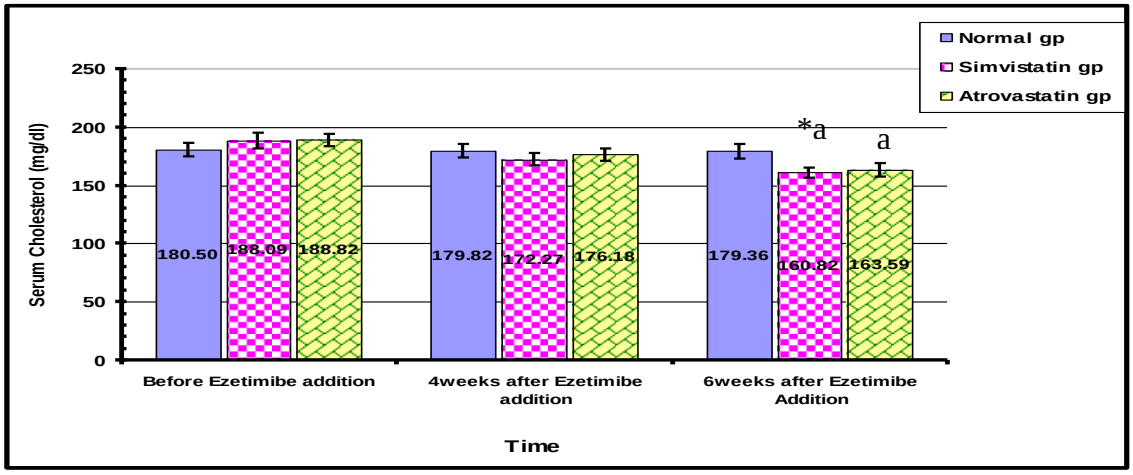

Figure (1) : A histogram showing serum total cholesterol, for Simvastain and Atrovastatin groups that received Ezitimibe; as compared with control

* = significant at $p<0.05$ as compare with normal values in same column.
a = significant at $p<0.05$ as compared with baseline values.

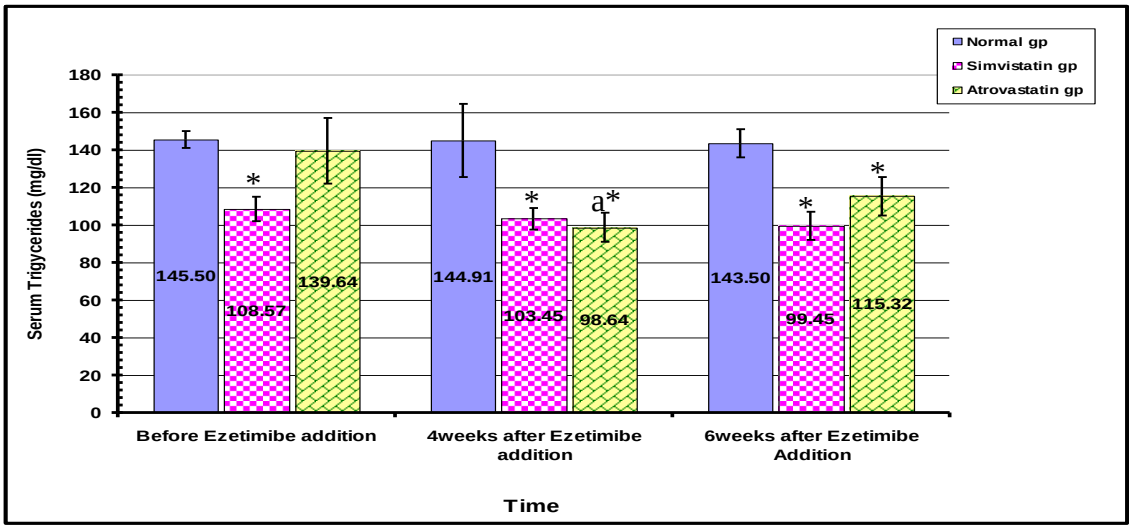

Figure (2): A histogram showing serum triglyceride, for Simvastain and Atrovastatin groups that received Ezitimibe; as compared with control group

* = significant at $p<0.05$ as compare with normal values in same column.
a = significant at $p<0.05$ as compared with baseline values.

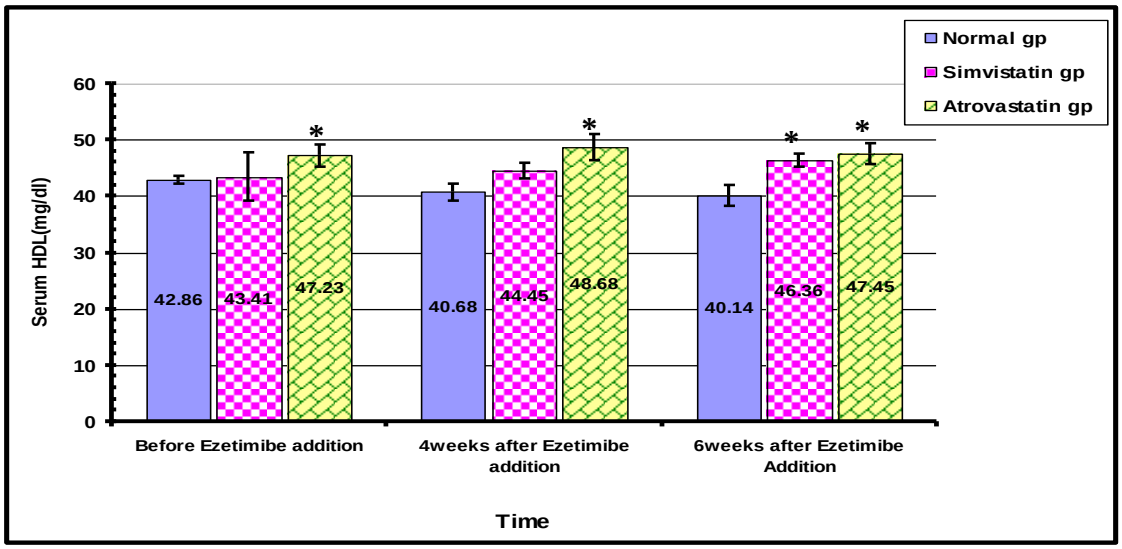

Figure (3): A histogram showing serum HDL-cholesterol, for Simvastain and Atrovastatin groups that received Ezitimibe; as compared with control group.

* = significant at p<0.05 as compare with normal values in same column.

But after 6 weeks there were significant (p<0.05) elevations in serum HDL-C level (15.4%,6.79%) as compared with normal and baseline values respectively .Atorvastatin group, showed significantly (p<0.05) higher serum HDL-C level before, after 4 weeks, and after 6 weeks from addition of ezetimibe. (10.19%, 19.66% & 18.21% respectively) as compared with value of normal group. Plasma LDL-C level was non significantly lowered after 4 weeks from addition of ezetimibe to simvastatin treated group (figure-4) but after 6 weeks there were a significant (p<0.05) lowering in serum LDL-C levels (-20.95%,-21%) as compared with baseline and normal values respectively. While, atorvastatin group, showed no significant alteration in serum LDL-C level both after 4 and 6 weeks from addition of ezetimibe. However, simvastatin group showed no significant changes in LDL/HDL ratio after 4 weeks of ezetimbe therapy as compared to those values before adding ezetimbe and that of control (table .2) .

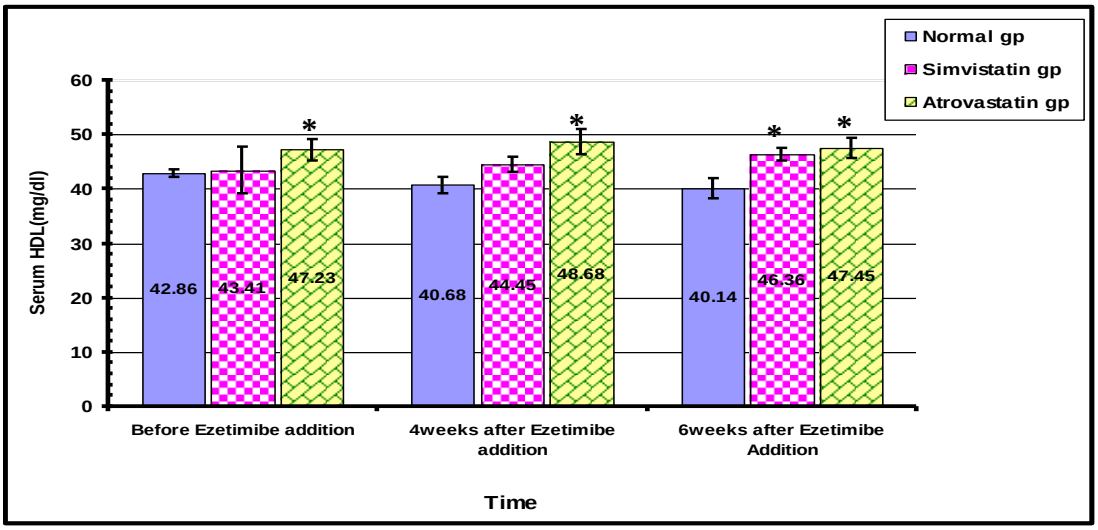

Figure (4) : A histogram showing serum LDL-cholesterol, for Simvastain and Atrovastatin groups that received Ezitimibe; as compared with control group.

* = significant at p<0.05 as compare with normal values in same column.

Table (2): Effect of Ezitimibe Addition on LDL/HDL ratio in patients treated with HMG-CoA reductase inhibitors (Atrovastatin & Simvastatin); in comparison with normal individuals (values expressed as mean ± standard error of mean , N= Number of subjects)

Groups	Duration of treatment	LDL/HDL ratio			
Normal N=22	Base line values	2.79	±	0.20	
	After 4 weeks	3.13	±	0.34	
	After 6 weeks	3.23	±	0.30	
Patient treated with Simvastatin & Ezitimibe N=22	Base line values	3.38	±	0.43	
	After 4 weeks	2.51	±	0.23	
	After 6 weeks	2.09	±	0.15	* a
Patient treated with Atrovastatin & Ezitimibe N=22	Base line values	2.68	±	0.22	
	After 4 weeks	2.35	±	0.24	
	After 6 weeks	2.34	±	0.19	*

* = significant at $p<0.05$ as compare with normal values in same column.
a = significant at $p<0.05$ as compared with baseline values.

But 6 weeks values were significantly ($p<0.05$) lowered (-38.16%,-35.29%) as compared with both baseline and control values respectively, a comparable results were obtained with atorvastatin treated group in LDL/HDL ratio (-27.5%,-12.68%)) as compared with normal and baseline values, respectively.In table -3, the studied groups exert a significant changes in serum ALT activity after 6weeks from addition ezetimibe (18.77%,5.66%)as compared to both baseline and control values respectively.While, the atorvastatine group exert no significant alterations in ALT activity in serum through the study. Simvastatin treated group of patients showed non significant elevation in serum AST activity after 4 weeks from addition ezetimibe(8.39%,11.7%) as compared with baseline and normal values ,respectively ,but after 6 weeks a significant ($p<0.05$) elevation in serum AST activity was noticed (13.6%)as compared to pretreatment value. Meanwhile, atoravastatin treated group was presented with a significant ($p<0.05$) lowering in serum AST activity (-7.12%,-14.4%)as compared with both baseline and normal values respectively. In simvastatin treated patients there was a significant ($p<0.05$)lowering in serum total bilirubin level(-15.5%,-17.09%) after4&6 weeks as compared to the control values, respectively (table-3). In atorvastatin treated group, ezetimibe showed a significant ($p<0.05$)lowering in serum total bilirubin (-8.18%, -13.6%) respectively as compared with baseline and normal values respectively.

Table (3) : Effect of Ezetimibe addition on serum ALT , AST and Total bilirubin in patients treated with Simvastatin and Atrovostatin in comparison with normal Subjects (values are expressed as mean ±SEM , N= Number of subjects)

Groups		Duration of treatment	Serum ALT (IU/L)				Serum AST IU/L	Serum total Bilirubin
Normal N=22		Base line values	13.82	±	0.72		14.8±0.66	1.26±0.03
		After 4 weeks	13.77	±	0.55		13.86±0.58	1.16±0.5
		After 6 weeks	13.59	±	0.47		14.50±0.65	1.17±0.04
Patient treated with Simvastatin & Ezitimibe N=22		Base line values	12.09	±	0.81		11.32±0.32*	1.03±0.04*
		After 4 weeks	14.18	±	0.66		12.27±0.48*	0.98±0.03*
		After 6 weeks	14.36	±	0.56	a	12.86±0.43a*	0.97±0.04*
Patient treated with Atrovastatin & Ezitimibe N=22		Base line values	12.91	±	0.60		12.77±0.56*	1.10±0.05*
		After 4 weeks	13.45	±	0.50		11.86±0.54*	1.03±0.05
		After 6 weeks	13.45	±	0.36		11.45±0.46*	1.01±0.03*

* = significant at $p<0.05$ as compare with normal values in same column.
a = significant at $p<0.05$ as compared with baseline values.

(Table- 4) showed no significant alteration in serum urea level before and after 4 and 6 weeks from addition of ezetimibe to all studied groups, nor in serum creatinine levels ,nor in microalbuminuria values. Serum MDA levels simvastatin treated groups were presented with a significant ($p<0.05$) lowering in serum MDA (-42%) as compared with baseline values (figure-5) .After 4 and 6 weeks of utilizing ezetimibe in atrovastatin treated group serum MDA significantly ($p<0.05$) lowered (19.8%,26.7%) as compared with baseline values.The simvastatin and atorvastatin treated patients showed no significant alterations in bleeding time values by the addition of ezetimibe to their therapy (table- 5).

Table (4) : Effect of Ezetimibe addition on serum urea,creatinine and microalbuminuria in patients treated with Simvastatin and Atrovostatin in comparison with normal Subjects (values are expressed as mean±SEM , N= Number of subjects)

Groups	Duration of treatment	Serum Urea(mg/dl)				Serum Creatinine (mg/dl)	Microalbuminuria (mg/day)
Normal N=22	Base line values	39.00	±	1.61		1.19±0.04	203.23±8.36
	After 4 weeks	39.73	±	2.03		1.16±0.05	207.09±11.3
	After 6 weeks	37.91	±	1.01		1.24±0.10	202.09±8.66
Patient treated with Simvastatin & Ezitimibe N=22	Base line values	39.43	±	1.98		1.28±0.14	201.73±8.59
	After 4 weeks	40.00	±	2.09		1.31±0.15	204.09±14.80
	After 6 weeks	39.27	±	1.78		1.29±0.14	206.09±18.70
Patient treated with Atrovastatin & Ezitimibe N=22	Base line values	36.68	±	2.08		1.13±0.04	211.55±10.40
	After 4 weeks	35.23	±	1.72		1.03±0.05	208.55±11.30
	After 6 weeks	36.23	±	0.97		1.11±0.02	209.05±17.40

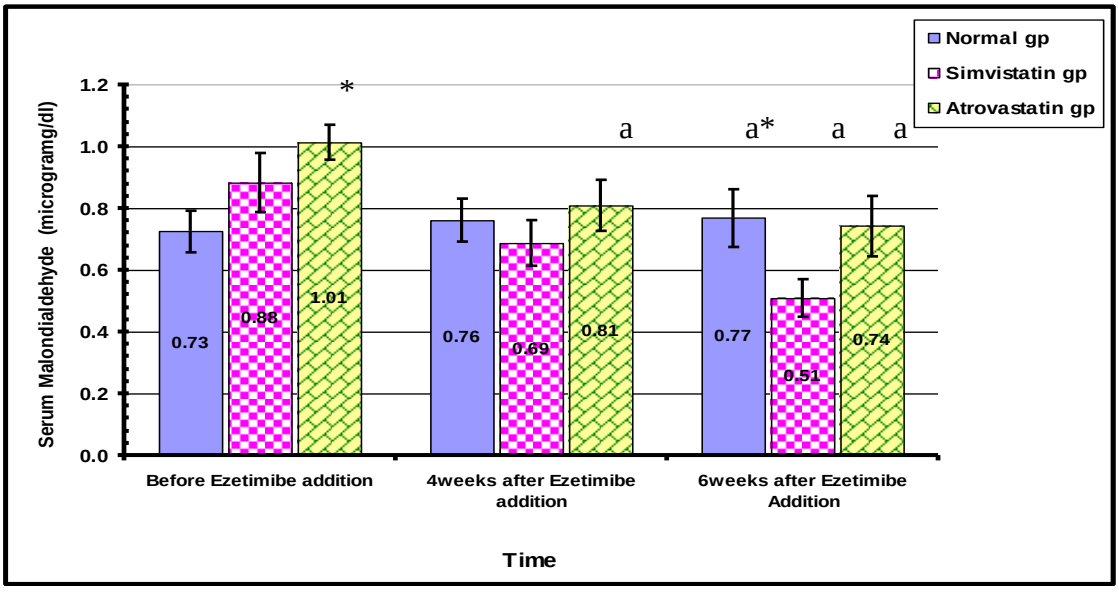

Figure (5): A histogram showing serum Malondialdehyde for Simvastatin and Atrovastatin groups that received Ezitimibe; as compared with control group

a = significant at $p<0.05$ as compared with baseline values.

* = significant at $p<0.05$ as compare with normal values in same column.

Table (5): Effect of Ezitimibe Addition on bleeding time, in patients treated with HMG-CoA reductase inhibitors (Atrovastatin & Simvastatin); in comparison with normal individuals (Values expressed as mean ± Standard error of mean , N= Number of subjects)

Groups	Duration of treatment	Bleeding time (minutes)			
Normal N=22	Base line values	2.54	±	0.18	
	After 4 weeks	2.48	±	0.17	
	After 6 weeks	2.45	±	0.13	
Patient treated with simvastatin & Ezitimibe N=22	Base line values	2.26	±	0.12	
	After 4 weeks	2.50	±	0.23	
	After 6 weeks	2.62	±	0.33	
Patient treated with atrovastatin & Ezitimibe N=22	Base line values	2.26	±	0.20	
	After 4 weeks	2.97	±	0.35	
	After 6 weeks	2.17	±	0.35	

Discussion

As previously presented in figures (-1), (-2), (-3) and (-4) treatment with ezetimibe plus statins successfully improves lipid profile markers in dyslipidaemic patients during 6 weeks of treatment .These results are consistent with results of other studies that included the administration of ezetimibe plus statins to patients with disordered lipids profiles could result in significant reduction in TC, LDL-C and TG levels, [36] with significant elevation in HDL-C levels , which could be attributed to mechanisms that are related to ezetimibe lowering effect on cholesterol which could be complement to the inhibitory action of statins on cholesterol biosynthesis representing an important new option for treatment in combination with statin [37]. Ezetimibe has an excellent safety and liability profile when administered with statins [38,39]. Also it has a low potential for drug interactions. [40] Many patients receiving statins therapy fail to reach their LDL- goal [41] , because its mechanism of action is complementary to that of statins, ezetimibe were studied for its potential additive lipid-lowering effects in patients already receiving statin therapy in double-blind as well as placebo-controlled trials [36,42]. Significant improvements were observed for other indicators of CHD risk (total cholesterol , non-HDL-C, apolipoprotein B, LDL-C: HDL-C ratio) in patients receiving ezetimibe-statin therapy.[36]When ezetimibe was combined with simvastatin or atorvastatin it caused a significant reduction in triglyceride level with time from baseline compared with statin monotherpy [43]. Combination therapy of simvastatin and ezetimibe was more effective than atorvastatin in reducing LDL-C in patients with primary hypercholesterolemia.[44] Preliminary studies have indicated that there were no significant effect of ezetimibe on absorption of fat-soluble vitamins[45] .Following absorption of ezetimibe where it is glucuronidated in the intestine wall the parent drug and its glucuronidated derivatives can undergo enterohepatic recircalation, that limits peripheral exposure [46] .Ezetimibe is first in cholesterol absorption inhibitors, it 's action is consistent with the binding thereby blocking of sterol transporter on the brush border membrane of in intestinal epithelial cells [47]. Through inhibiting the intestinal cholesterol absorption ezetimibe can effectively reduce of biliary/dietary cholesterol delivered to the liver via chylomicron and chylomicron reminants, hence reduce cholesterol content of atherogenic particles chylamicrons / chylomicrors ruminants, VLDL, LDL).Meanwhile the reduced delivery of intestinal cholesterol to liver increase hepatic receptor activity and increase clearance of circulating LDL-C. [47].Ezetimibe, via inhibiting intestinal cholesterol and plant-sterol absorption, may modify the atherogenicity of chylomicron remnants and reduce systemic plant-sterol levels[48]. These effects are likely to reduce cardiovascular risk. It has been reported that there is a strong relationship between hepatic dysfunction and dyslipidaemic complications[49,50] However, the data presented in table- 3 representing a modulation in some liver markers in group treated with simvastatin plus ezetimibe after 6 weeks of treatment in case of ALTand AST. Such results could be due to relatively low doses of statins (20mg) wherase, other studies revealed a significant elevation in those enzymes.[13]The elevation in transaminases activities were primarily asymptomatic and not

associated with cholestasis. Serum transaminases returned to pretreatment level with discontinuation of combination therapy or with continued treatment [51] .Ezetimibe is being used with increasing frequency in many patients to augment the LDL-cholesterol lowering effects of statins [52] The recent Second United Kingdom Heart and Renal Protection (UK-HARP-II) study found in a randomized, controlled study that 10mg of ezetimibe added to 20mg of simvastatin in patients with (chronic kidney disease) CKD resulted in an incremental reduction of LDL-cholesterol level over simvastatin alone without an excess risk of abnormal liver or muscle markers or other advers events. [53] The purpose for the evaluation of renal function was to explor the safety of a combination of ezetimibe and statins in this respect .Statins at appropriately adapted doses have the same efficacy in chronic renal disease patients as in subjects with normal kidney function, and their tolerance is not a problem.[54] In the present study the effect of ezetimibe plus statins (simvastatin or atorvastatin) have no significant effect on renal function , as in table-4 . Therefore, no dosage adjustment for ezetimibe is needed in patients with renal insufficiency. Efforts to improve lipid profiles now are targeted primarily for the treatment and prevention of cardiovascular disease, may also prevent the development of renal disease [55] .One important risk factor for atherosclerosis is an elevation in a particular type of plasma cholesterol specifically LDL -C. Oxidation of LDL -C is thought to render the lipoprotein to be athergenic ,because oxidized -LDL is more readily taken up by macrophages via scavenger receptors. [56]The data presented in figures (5) showed that serum MDA levels were significantly lowered by about (-33.7%) when ezetimibe was added to simvastatin treated group after 6 weeks .Meanwhile, level of MDA was significantly lowered after 4 weeks from addition ezetimibe to atorvastatin treating group by about (-19.8%) as compared with pretreatment values . However, MDA level after 6 weeks from addition ezetimibe to either groups lowered MDA level below those reported even for the normal group . Furthermore , the lowering effect on lipids pereoxidation produced by simvastatin/ezetimibe combination was better than that produced by ezetimibe/atorvastatin combination.[57] A recent study showed that despite the comparable modest reduction of serum cholesterol levels by ezetimibe, an intestinal inhibitor of cholesterol absorption, and statin, only the statin improved endothelial function[58] Thus, it is likely that the beneficial effects of statins on endothelial function extend beyond cholesterol reduction. Indeed, statins have been shown to reduce cardiovascular events in patients, irrespective of serum cholesterol levels [59] . In this study, the effects of adding ezetimibe to statins therapy (Simvastatin or Atorvastatin), in patients with dyslipidemia showed no significant changes in platelets function in both groups, this could indicate no adverse effect on platlets function as seen in table -5 .Although non of the studied groups of dyslipidaemic patients exert any deviation in bleeding time values from those reported from normal subjects ,before initiating ezetimibe therapy. This would support the administration ezetimibe plus simvastatin or atorvastatin without any adverse effect on platelets, so patients with platelets dysfunction could take ezetimibe with statin safely .However, there is no evidence or clinical trials about effect of ezetimibe with statins on platelets function and specifically on bleeding time.

conclusions:

1. Ezetimibe can be used in combination with simvastatin or atorvastatin to improve their lipid-lowering action both effectively and safely in the treatment of dyslipidemia.
2. Ezetimibe can be used safely in combination with statins in patients with renal disease.
3. Ezetimibe exerts no further modification to liver function that could be produced by statins when used in combination with statins.
4. Ezetimibe/simvastatin and Eztimibe / atorvastatin could exert asignificant antioxidant effect in patients with dyslipidemia.
5. Non of the tested drugs (ezetimibe nor simvastatin nor atorvastatin) produced significant modification of platelets activity.

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In Situ Gelling Formulation of Naproxen for Oral Sustained Delivery System

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Abstract

Naproxen is non-steroidal anti-inflammatory drug, which has antipyretic and anti-inflammatory effect. It is extensively bound to plasma albumin, and exhibits gastric toxicity, so it may be more efficient to deliver the drug in its sustained release dosage form and adequate blood level is achieved. Three liquid formulations with in situ gelling properties have been assessed for their potential for the oral sustained delivery of naproxen . The formulations were dilute solutions of: (a) pectin; (b) gellan gum and; (c) sodium alginate, all containing complexed calcium ion that form gels when these ions are released in the acidic environment of the stomach . The viscosity of the sols and drug release were measured, and was found to be dependent on the type and concentration of the gelling agent. Pectin sol shows the highest viscosity and drug release . The influence of variation of gastric pH and the effect of added 1.6 mM Ca^{++} ions on the gelation property and the release profile of the liquid formulations were examined. The efficiency of gelation was significantly reduced with increase of pH. In addition the influence of different concentrations of sorbitol were determined .The results showed that 10% w/v sorbitol is the best concentration that maintained fluidity and ease of administration for the selected formula . The selected formula was examined for its stability and expiration date, and, it was found that there was no evidence of physical changes under experimental conditions, with estimated expiration of about 4.1 years and pH of the formula stated at 5.1.

Key word: naproxen, in situ gelling, oral preparations, gel.

الخلاصة

النابروكسين هو عقار غير ستيرويدي مضاد للالتهابات و خافض للحرارة ذو اعراض مخدشة للمعدة و طعم غير مستساغ و كذلك يرتبط بشدة مع البومينات البلازما , لذا قد يكون اكثر كفاءة اعطاء العقار على شكل جرعة دوائية مديدة المفعول للحصول على مستوى كافي منه في الدم . ثلاث تراكيب سائلة ذو صفة التحول الى هلام في الموقع قد تم تحليل قابليتها على التحرير الفموي المديد المفعول للنابروكسين. التراكيب كانت محاليل مخففة من: (ا) البكتين (ب) جيلان كم (ج) الجينات الصوديوم, و كلها تحتوي على ايونات الكالسيوم المعقدة و التي تكون الهلام عندما تتحرر هذه الايونات في الوسط الحامضي للمعدة. لقد تم دراسة لزوجة المحلول الروي و تحرر الدواء منه, ووجد بانه يعتمد على نوع و تركيز المادة الهلامية , فالمحلول الغروي للبكتين اظهر اعلى لزوجة و تحرر للدواء. لقد تم دراسة تاثير اختلاف حامضية المعدة و تاثير ايونات الكالسيوم المضافة (١,٦ ملي مول)على خاصية تكون الهلام وكذلك الية تحرر الدواء. ان فعالية تكون الهلام قد قلت بشكل واضح مع ازدياد الاس الهيدروجيني. بالاضافة الى ذلك فقد تم تحديد تاثير التراكيز المختلفة لمادة السوربيتول . فقد اظهرت النتائج بان تركيز السوربيتول ١٠٪ هو افضل تركيز لانه يحافظ على سيولة و سهولة استعمال التركيبة المختارة.تم دراسة ثباتية التركيبة المختارة و تاريخ انتهاء الصلاحية ووجد بانه ليس هناك تغييرات فيزياوية تحت ظروف التجربة مع تاريخ انتهاء صلاحية يقدر ب ٤,١ سنة و حامضية للتركيبة ثابتة على ٥,١ .

Introduction

Solutions that undergo sol-gel transformation when they meet physiological conditions may serve as an in situ gelling drug delivery system [(1)]. In situ is a Latin phrase meaning in the place. The new concept of producing a gel in situ was suggested for the first time in the early 1980s. It is widely accepted that increasing the viscosity of a drug formulation in the precorneal region will lead to an increased bioavailability, due to slower drainage [(2)].Gels are transparent or translucent, non-greasy, semisolid preparations. These are also termed jellies consisting of either suspensions made up of small inorganic particles, or large organic molecules interpenetrated by a liquid [(3)].Hydrogel is three-dimensional hydrophilic polymeric networks capable of imbibing large quantities of water have generated a lot of interest recently as delivery system for pharmaceutically active agents [(4)]. One of the main characteristics of hydrogels is that they contain ingredients that are dispersible as colloids or are water-soluble [(5)].The swelling of environmentally sensitive hydrogel can be affected by many stimulus, these are: temperature, pH, ionic concentration, electrical field, inflammation, solvent concentration, light and radiation, magnetic field and glucose concentration [(6)]. According to the mechanism by which sol - gel phase transition occur, the following three types of systems can be recognized [(7)]:

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1- pH triggered systems.
2- Temperature sensitive system.
3- Ion activated system.

From the point of view of patient acceptability, a liquid dosage form that can sustain drug release and remains in contact for extended period of time, improving the bioavailability, reducing the dose concentration and frequency may be achieved by in situ gelling formulations [8].Gelation of the orally administered liquid formulations (Ion activated system) was ensured by the inclusion of calcium ions in the formulation as a soluble complex designed to break down to release free calcium ions on encountering the acidic environment of the stomach [9]. The gelation was delayed until the orally administered solution reached the stomach by complexing the calcium with sodium citrate [10].Naproxen is a non steroidal anti-inflammatory drug (NSAID) advocated for use in painful and inflammatory rheumatic arthritis, osteoarthritis, migraine, postoperative pain and postpartum pain [11]. The goal of this study is to prepare liquid formulation of naproxen with in situ gelling properties and assessed for its potential sustained oral delivery system.

Experimental

Materials and Equipments

Naproxen, Methyl paraben, Propyl paraben (Supplied by Samarra drug industries, Iraq). Amrixen® suspension (Amrit Medical Co.). Calcium chloride, Disodium hydrogen phosphate, Potassium dihydrogen phosphate (BDH chemical Ltd.pool, England). Cellulose membrane (Viskase Sale Co., size 36/32, USA). Gellan gum (Dainippon pharmaceutical Co., Osaka). Hydrochloric acid, Sorbitol (Riedel-de haen Hannover, Germany). Pectin (Cesalpinla food com., Italy). Sodium alginate (Hopkin and Williams Ltd, England).Diffusion cell (Plastic dialysis cell, modified Franz cell), pH meter (Hanna instrument pH211, Italy), UV spectrophotometer (Carrywin UV, Australia), Viscometer (Brookfield DV-II , England).

Method of Preparation

Preparation of the Sols:

Sodium alginate and pectin solution of concentrations 1.0, 1.5 and 2.0 % (w/v) were prepared by adding the polymer to distilled water containing 0.25% (w/v) sodium citrate and 0.075% (w/v) calcium chloride and heating to 60°C for sodium alginate and 40–50 °C for pectin while stirring. Naproxen equivalent to 2.5% (w/v) was then dispersed in the resulting solution after cooling to below 40°C [12, 13]. Gellan gum solutions of concentrations 0.25, 0.5 and 1.0% (w/v) were prepared by adding the gum to distilled water containing 0.17% w/v sodium citrate and heating to 90°C while stirring. After cooling to below 40°C appropriate amounts of calcium chloride 0.016% (w/v) and 2.5% (w/v) naproxen were then dispersed in the resulting solution [12].

Gelation Property

Instantaneous gelation was checked by addition of the sols dropwise to simulated gastric fluid pH 1.2 [14].

The Effect of Different Concentrations of Calcium Chloride and Sodium Citrate on the Gelling Properties:

The optimum quantities of calcium chloride and sodium citrate that maintained fluidity of the formulation before administration and resulted in gelation when the formulation was added to simulated gastric fluid, were determined by preliminary tests in which pectin sols 1%, (w/v) containing sodium citrate concentrations of 0.125, 0.25 and 0.50% (w/v) and calcium chloride concentrations of 0.05, 0.075 and 0.1 % (w/v) were added dropwise to 50 ml simulated gastric fluid (pH1.2).

Measurement of the Rheological Properties of Sols:

The viscosity of sols prepared in water was determined at room temperature (25 ºC) with Brookfield Digital Viscometer [14].

Measurement of In Vitro Drug Release:

The release rates of naproxen were measured using plastic dialysis cells similar to that described previously by Miyazaki et al [15, 9]. The capacity of each half-cell was 4 ml and the surface area of the membranes was 2.67 cm^2.Sols of pectin, gellan gum or alginate were placed in the donor compartment individually. An equal volume of simulated gastric (pH 1.2) or intestinal (pH 6.8) fluid was placed in the receptor compartment. The donor phase and the aqueous receptor phase were separated by a cellulose membrane. The assembled cell was shaken horizontally at rate of 60 strokes per min. in an incubator maintained at 37°C temperature. The total volume of the receptor solution was removed at intervals and replaced by fresh release medium. The drug concentration of the samples was determined using UV spectrophotometer.

Effect of pH and Added Ca^{++} Ion on:

The gelation:

The influence of pH on the gelation characteristics of 1% (w/v) pectin sols was determined by immersion of 30 ml sol enclosed in cellulose membrane tubing into simulated gastric fluid (150 ml) with pH values range 1.0–5.0. After equilibration for 24

hr at room temperature, the contents of the tube were passed through a sieve (No. 6.5, 2.80 mm) over a period of 30 seconds and the weight of the gel remaining in the sieve was determined by balance. The experiments were repeated in the presence of added 1.6 mM Ca^{++}[16].

In vitro release:

- The effect of pH:

The in vitro release of naproxen from 1.0% (w/v) pectin was measured using an equal volume of simulated gastric (pH 1.2 and 3.0) for 1 hour and intestinal (pH 6.8) fluid for 5 hours placed in the receptor compartment[17].

- The effect of Ca^{++}:

The release measurement was done at pH 3 using 1.0% (w/v) pectin sol alone and with added 1.6 mM Ca^{++} [16].

Effect of Different Concentrations of Sorbitol on:

Rheological properties:

Different concentrations of sorbitol (0, 5, 10, 20, 30, and 40% w/v) were added to 2.0% (w/v) pectin sols loaded with 2.5% (w/v) naproxen, and the viscosities were measured[18].

In vitro release:

The in vitro release was measured for 2.0% (w/v) pectin sols loaded with 2.5% (w/v) naproxen, in presence of different concentrations of sorbitol (0, 5, 10 and 20% w/v) [18].

Stability Study:

Several glass containers (each containing 4 ml) of the selected formula were incubated at 35, 50 and 60°C for 90 days. Samples were taken at specified time intervals and assayed for their drug content. The physical appearance and the pH of the formula were also evaluated.

Results and Discussion

Gelling Property

In this study Ca^{++} ions were included in all formulations for induction of gelation. However, for ease of administration the prepared formula must be introduced in a fluid (sol) state. This was achieved by addition of sufficient sodium citrate to the formulation to form a complex with all of the Ca^{++} ions present in the formulation and hence to effectively remove them from solution. Then, in the acidic environment of the stomach the complex is broken down and the Ca^{++} ions released cause gelation to occur [19].Instantaneous gelation was observed by addition of the sols of pectin, sodium alginate and gellan gum dropwise to simulated gastric fluid maintained at pH 1.2.

The Effect of Different Concentrations of Calcium Chloride and Sodium Citrate on the Gelling Properties:

The results indicated that the minimum concentration that maintained fluidity of the sol before administration and caused gelation of sols in the gastric fluids was 0.25% (w/v) sodium citrate and 0.075% (w/v) calcium chloride. Moreover, gelation occurred without exposure to simulated gastric fluid pH 1.2 in formulations containing 0.050, 0.075 or 0.1% (w/v) $CaCl_2$ and sodium citrate concentration of 0.125% (w/v) as shown in table(1). The increase in calcium chloride content to 0.10% (w/v) with the same sodium citrate concentration caused gelation of the formulation before contact with simulated gastric fluid [13].

Table (1): The effect of different concentrations of sodium citrate and calcium chloride on the gelation of 1% (w/v) pectin sols before and after administration to simulated gastric fluid.

Sodium Citrate / Calcium Chloride	0.125% (w/v)	0.25% (w/v)	0.5% (w/v)
0.05% (w/v)	Gel before administration	Sol before administration Friable and soft gel after administration	Sol before administration Friable and soft gel after administration
0.075% (w/v)	Gel before administration	Sol before administration Optimal gel strength after administration	Sol before administration Low gel strength after administration
0.1% (w/v)	Gel before administration	Gel before administration	Gel before administration

Rheological Properties of the Sols:

All the prepared sols revealed that the viscosity is increased as a function of increasing polymer concentration with shear thinning behavior.The rheogram profiles of different polymers used in this study, suggested that pectin sols used is more accepted one than other two polymers (gellan gum and sodium alginate), since pectin sols exhibited more or less fit profile with that obtained from commercial one Amrixen ® suspension as a reference product. Moreover, the results indicated that best concentration required for incorporating pectin as a gelling agent is 1.5% (w/v) and to a lesser extent for 1.0% (w/v) concentration.

Dissolution behavior (In Vitro Release):

Large increment in the amount released of naproxen observed when the receptor solution was changed from simulated gastric fluid pH (1.2) to simulated intestinal fluid pH (6.8).This was expected since there will be change in the state of ionization of the acidic drug (pKa of naproxen is 4.2) accompanying the pH range. It is completely unionized at pH 1.2 and this lead to negligible drug release at this pH[20].Rigid gels are formed when the donor solutions of all systems are placed in contact with a receptor solution at pH 1.2 and, as a consequence, the amount of the drug released is lower than that at pH 6.8, which is referred to the high H^+ ion concentration at pH 1.2 that is sufficient to cause the formation of rigid gels [21]. There was a significant decrease in the release rate with increasing polymer concentration. This behavior may be attributed to the effect of mechanical barrier that set up by the random network of the polymer gel molecules which binds and entraps surrounding water. This aqueous phase in the polymer network acts as the region responsible for diffusion of the drug in the gel. The change of the polymer concentration of these gels could affect the diffusion pathway and thus the drug release [22]. In addition, as the viscosity of the polymer sols increased with concentration, the solvent penetration into the core of the matrix will be decreased, and the drug release will be decreased [23]. In an attempt to verify the effect of polymer types on the release of naproxen, the cumulative release profiles of 2.5% (w/v) naproxen from 1% (w/v) different gelling polymers were constructed as shown in figure (1).

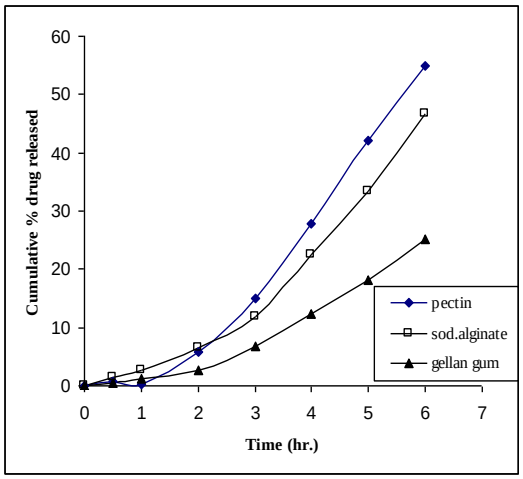


Figure (1): Cumulative in vitro release of naproxen (2.5% w/v) from 1% (w/v) concentrations of pectin, sodium alginate and gellan gum gels.

The results obtained indicated that the release of naproxen from different types of polymers was in the following order: pectin > sodium alginate >gellan gum. This suggests that the choice of the polymer base is of obvious importance for achieving a desired drug release.The explanation for this related to the diffusivity of the drug through any base depends on the nature and composition of individual base and the drug-vehicle interaction. Also, the solubility of the drug in the vehicle affects the drug release and diffusion [24].The release data over the whole time period were analyzed according to the treatment proposed by Higuchi for drug release from semisolid vehicles [25]. For the initial cumulative drug released 50-60%, the amount "Q" of drug released per unit surface area from gel is proportional to the square root of time:

$$Q = 2 C_o (D t / \pi)^{1/2}$$

In which Q is the amount of drug released per unit area; C_o the initial drug concentration in the vehicle; D is the diffusion coefficient of the drug in the matrix and t is the time.Plots of Q versus $t^{1/2}$ for the release of naproxen from all gels were linear after a short lag period indicative of diffusion controlled release [26] as shown in figure (2). There is usually a lag period until water permeates the polymer mass to create pores for diffusion of the drug .Later, the drug released [27].

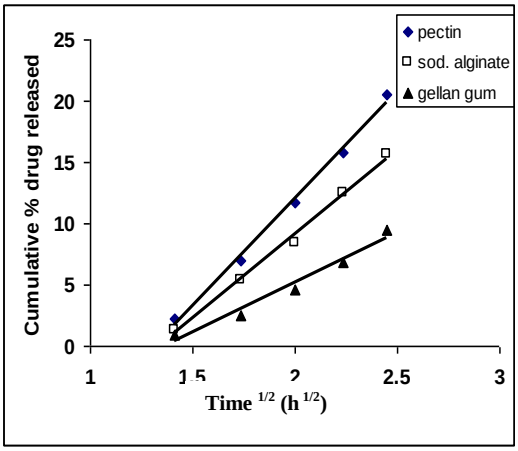


Figure (2): Cumulative % released of naproxen as a function of square root of time from 1% (w/v) concentrations of pectin, sodium alginate and gellan gum gels.

Effect of pH and Added Ca^{++} Ion on:

The gelation:

The results show that the hydrogen ion concentration at pH 1.0–2.5 was sufficiently high to cause gelation in the absence of an additional source of calcium. Visual observation showed well-defined compact gels over this pH range. Although complete gelation was observed at pH 2.5- 3.5, the resultant gels were not sufficiently strong to maintain their cylindrical form. However, at higher pH (pH >3.5) and when H^+ ions are insufficient, the effective breakdown of the calcium complex in the sols and gelation was poor. Figure (3) showed that the addition of 1.6 mM Ca^{++} ions was sufficient to cause almost complete gelation of formulations over the entire pH range examined. The gels formed at pH > 2 had a loose, less structured appearance.

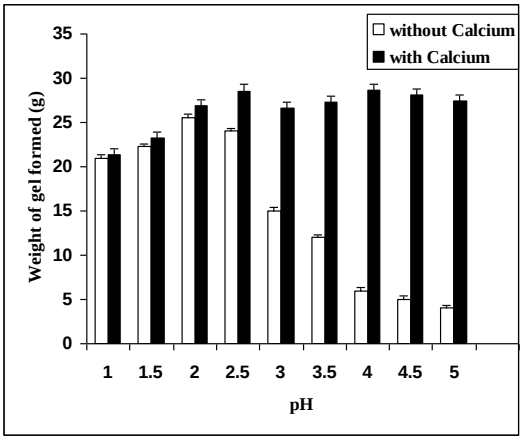
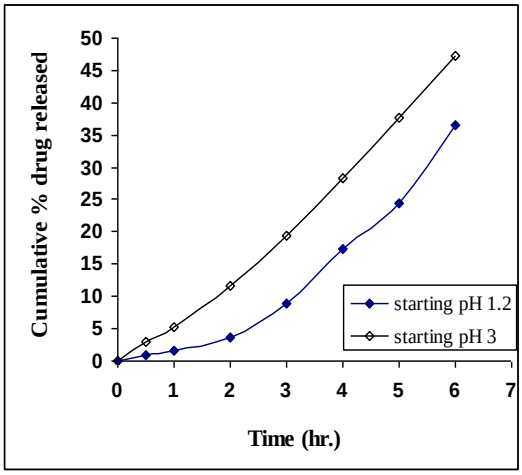


Figure (3): The effect of pH and added 1.6 mM Ca^{++} ions on the weight of gel formed from 30 ml solutions (1.0% (w/v) pectin as a function of pH. Each value is the mean ± S.E of 3 determinations.

In Vitro Release:

- The effect of pH:

The release of drug was appreciably faster when pectin was exposed to receptor solutions at pH 3.0 over the initial 1 h release period as shown in figure (4). Observation of the donor cells showed that the formulations were in sol form throughout the duration of the release period. Diffusion of H^+ ions from the receptor solution at this pH was insufficient to cause the release of complexed calcium ions and consequently gelation of the pectin was incomplete [17].



Figure(4): The effect of starting pH in the receptor solution on the release of naproxen from 1.0% (w/v) pectin gels.

-The effect of added Ca^{++}:

The influence of added 1.6 mM Ca^{++} in the formula on drug release from pectin formulations exposed to receptor solutions at pH 3.0 is shown in Figure (5). Observations of the donor cells showed the presence of a thin gel layer on the surface of the cellulose membranes when calcium was included in the formulation but no gelation of the bulk of the sol [16].

Effect of Different Concentrations of Sorbitol:

High concentration of pectin sol was used to study the effect of different concentrations of sorbitol, since it could withstand the effect of high concentrations of sorbitol.

Rheological properties:

Figure (6) shows the influence of sorbitol concentration on the flow properties of 2% (w/v) pectin sol. The viscosity of the pectin sols increased appreciably as the sorbitol concentration was increased from 5 to 40% (w/v).Addition of 5% and 10% (w/v) sorbitol to the sols caused a reduction of viscosity at all

shear rates. These changes in viscosity resulting from sorbitol addition, since it is hygroscopic and may withdrawn water to the gel structure that decreases viscosity and hence improving the ease of swallowing of the sols [28].A considerable increase of viscosity was noted with sorbitol concentrations between 20% and 40% (w/v), all formulations exhibiting a change of flow properties from shear thinning to Newtonian behavior [18].Sorbitol at higher concentrations binds with water molecules causing desolvation around the pectin chains and minimizing the hydrogen bonding of water molecules to pectin chains. As a consequence, pectin chains cross-linked together and result in increased viscosity [18].

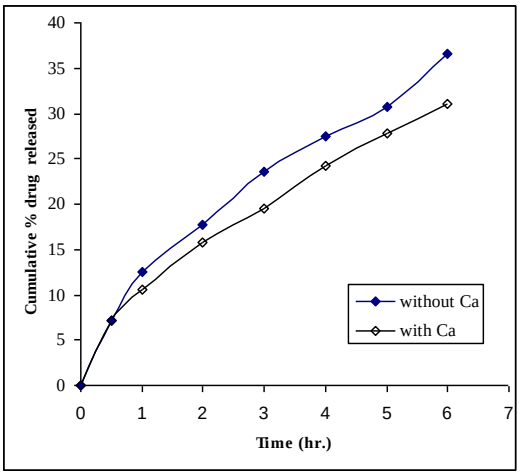
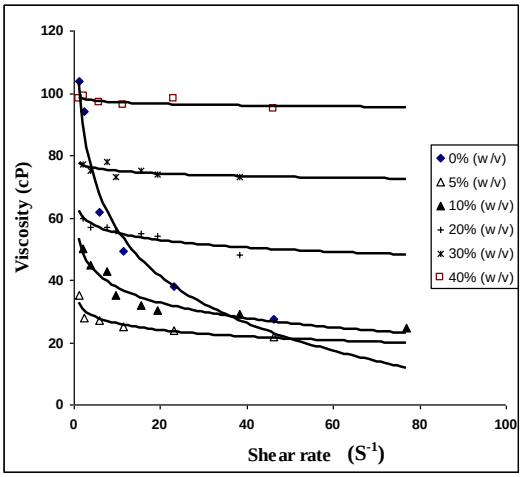


Figure (5): Effect of added Ca^{++} ions on the release of naproxen from 1.0% (w/v) pectin gels.



Figure(6): Effect of sorbitol concentration on the viscosity of 2.0% (w/v) pectin sols loaded with 2.5% (w/v) naproxen at 25 ºC.

In vitro release:

The release profiles of naproxen from gels of 2% (w/v) pectin containing sorbitol concentrations over the range 0–20% (w/v) was shown in figure (7). For gels containing 20% (w/v) sorbitol there was a pronounced increase of release after about 3 hours. No such inflection was observed for gels formed in the presence of 0, 5 and 10% (w/v) sorbitol. Observation of the contents of the donor cell during release measurements showed that the inflection in the plots for release from the formulation containing 20% (w/v) sorbitol coincided with a gel to sol transition, i.e. low gel strength to withstand a large decrease of hydrogen ion concentration; gels formed in formulations containing lower sorbitol contents retained their integrity throughout the measurement period [14].

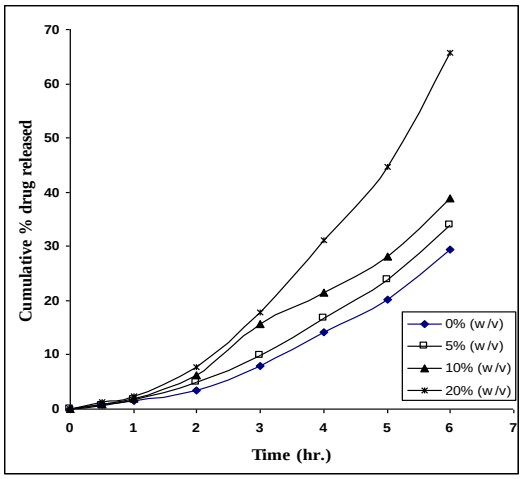


Figure (7): Effect of sorbitol concentration on the release of naproxen from 2.0% (w/v) pectin gels loaded with 2.5% (w/v) naproxen.

Stability Study of Selected Formula (Prediction of expiration):

The final formula of this study was introduced into an exaggerated temperatures study maintained at (35, 50 and 60 ºC) to predict expiration date.

$R_{/}$

Naproxen	2.5 gm
Pectin	1.5 gm
Sodium Citrate	0.25 gm
$CaCl_2$	0.075 gm
Sorbitol	10 gm
Methyl paraben	0.2 gm
Propyl paraben	0.02 gm
Dis. Water up to	100 gm

The degradation of naproxen in this formula followed first order kinetics since

straight lines were obtained by plotting the logarithm of the percent remaining of naproxen versus time as shown in figure (8). The first-order reaction equation:

$$\textbf{Log C = Log C}_o \textbf{ – K}_1 \textbf{ t / 2.303}$$

Where C_o is the initial concentration of naproxen, C is the remaining concentration at time t and K_1 is the first order rate constant. The slope of the line is $– K_1 /2.303$ from which the rate constants obtained. Table (2) shows the degradation rate constants of naproxen at different temperatures . To determine the expiration date (t_{10} %), Arrhenius plot was constructed to predict the degradation rate constant of naproxen at 25 ºC as shown in figure (9).The expiration date of naproxen in the suggested formula was calculated according to the first order reaction equation:

$$t_{10\ \%} = 0.105 / K_{25\ ºC}$$

The expiration date was found to be equal to 4.1 years with pH of 5.1 for whole the period.

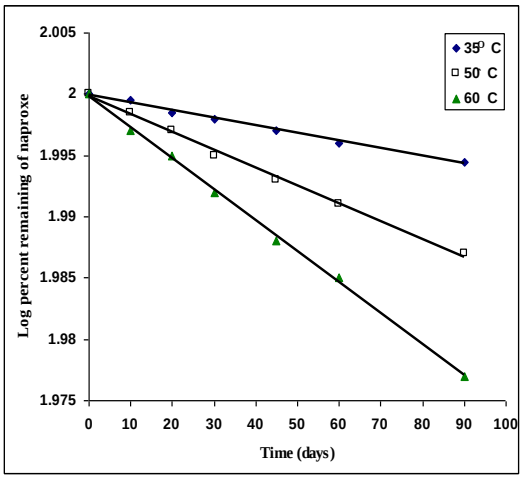


Figure (8): Accelerated stability study of naproxen in the selected formula at elevated temperatures (35, 50 and 60 ºC).

Table (2): Degradation rate constants (K) of naproxen sol at different temperatures.

Temperature	35 ºC	50 ºC	60 ºC	25 ºC
K (day)$^{-1}$ ×10^{-4}	1.545	3.97	6.91	0.695

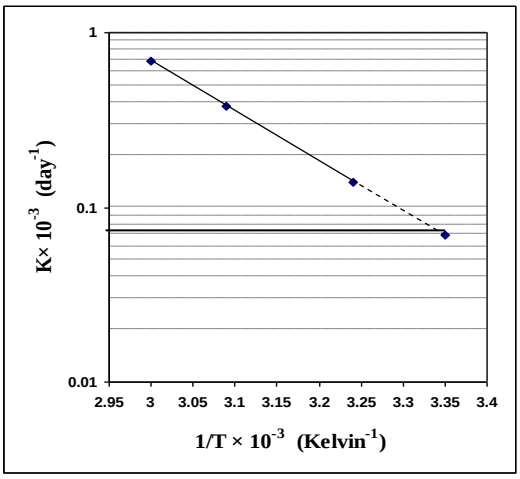


Figure (9): Arrhenius plot for expiration date estimation of naproxen in the selected formula.

Conclusions

Based on the results obtained, the optimum concentration of calcium chloride was 0.075% (w/v) and sodium citrate was 0.25% (w/v) for in situ gelling formulations of naproxen. The gelation, viscosities of the sols and the in vitro release of naproxen from the gels were affected by the type and concentration of the gelling agent, initial loading of naproxen, and concentration of sorbitol in the formula, gastric pH and added Ca^{++} ions. The most promised selected formula was pectin-gel type, with stable physical properties maintained at pH 5.1 and 4.1 years shelf life.

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The Safety Profile of Single Daily Dose of Aminoglycosides in Comparison with Multiple Daily Dose

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Abstract

To overcome the problems which associated with the standard multiple daily doses (MDD)
of aminoglycosides (AGs) like high incidence of toxicity(nephrotoxicity, ototoxicity)(5-25%) and high cost, an alternative approach was developed which was single daily dose (SDD).This new regimen was designed to maximize bacterial killing by optimizing the peak concentration/minimum inhibitory concentration(MIC)ratio and to reduce the potential for toxicity. The study includes 75 patients selected randomly, 50 of them received SDD regimen of age range of 17-79 years and the remaining received MDD regimen of age range of 13-71 years. The study was designed to evaluate the safety of SDD regimen in comparison with MDD regimen. All the patients in SDD group received a constant dose of 5-mg/kg/day of gentamicin and 20mg/kg/day of amikacin with a drug administration interval based on estimated creatinine clearance(CLcr): if ≥60 ml//min every 24 hours (q24h), 59- 40 ml/ min every 36hours and 39- 30 ml/min every 48 hours.The calculated dose was diluted with 0.9% normal saline or 5% dextrose to 50-100 ml and given as intravenous infusion over 30-60 minutes. In SDD group , the mean length of therapy was 6.4±1.73 days .Gentamicin accounted for 96% of the aminoglycoside use, and the majority(58%) of patients received the drug every 24 hours.The 36- and -48 hours intervals were used for 34 and 8% of the population, respectively.While in MDD group , the mean length of therapy was 5.0±0.91 days. Gentamicin accounted for all (100%) of aminoglycoside use, and all of the patients received the drug every 8 hours. No clinically apparent ototoxicity and nephrotoxicity were observed in the patients in the SDD group, in contrast to the patients in MDD group, in whom 4 patients (16%) were developed nephrotoxicity and 1 patient (4%) was developed ototoxicity. The obtained results indicate that SDD regimen was safer through decreasing the incidence of both nephrotoxicity and ototoxicity.For statistical analysis, ANOVA test was used with $P<0.01$.Each mean was expressed as mean±SEM(Standard Error of Mean).

Key words: Aminoglycosides, Single Daily Dose, Nephrotoxicity and Ototoxicity.

الخلاصة

من اجل تجاوز المشاكل المصاحبة للجرع القياسية المتعددة من دواء الامثيوكلاكوسايد بسبب السمية العالية كتلف الكلى والاذن (5- 25%) والكلف العالية . استحدثت دراسة بديلة في استخدام نظام الجرعة اليومية الواحدة هذا النظام الجديد صمم لزيادة القابلية القاتلة للبكتريا عن طريق زيادة نسبة قيمة التركيز / اقل تركيز مثبط والاقلال من الاعراض الجانبية . تشمل الدراسة على 75 مريض ثم اختيارهم عشوائياً ضمنهم 50 مريض كانوا باعمار (17 – 79) سنة استلموا الدواء بنظام الجرعة الواحدة , العدد المتبقي كان بمعدل الاعمار (13 – 71) سنة استلموا الدواء بنظام متعدد الجرع . صممت الدراسة لتقييم سلامة نظام الجرعة الواحدة بالمقارنة بنظام متعدد الجرع جميع المرضى في نظام الجرعة الواحدة استلموا جرعة ثابتة 5 ملغم /كغم /يوم من مادة جنتمايسين و 20 ملغم /كغم /يوم من مادة امي كيسين مع فاصل اخذ العلاج اعتماداً على وضوح الكرياتينين المخمن اذا كان اقل من 60 مل / دقيقة يعطى الدواء كل 24 ساعة , 59 – 40 مل / دقيقة كل 36 ساعة و 39 -30 مل / دقيقة كل 48 ساعة . الجرعة المسموحة خففت بمحلول الملح الطبيعي 0.9 % او محلول الدكستروز 5% الى 50 – 100 مل واعطي عن طريق النضوح الوريدي خلال 30 – 60 دقيقة في نظام الجرعة الواحدة كان معدل طول فترة العلاج 6.4 ± 1.73 ايام تم اعطاء الجنتامايسين الى 96% من المرضى والغالبية منهم (58%) استلمو الدواء كل 24 ساعة فواصل الـ 36 و48 ساعة استخدم لـ (34%) (8%) من المرضى بالتوالي . بينما في نظام متعدد الجرع كان معدل طول فترة العلاج 5 ± 91 ,0 ايام جميع المرضى (100%) استلموا جنتامايسين كل 8 ساعات . لايوجد ظهور سريري في تسمم الاذن والكلى عند المرضى المستخدمين لنظام الجرعة اليومية الواحدة . بينما في نظام متعدد الجرع حيث ظهرت 4 حالات تلف (16 %) كلى وحالة تلف تسمم الاذن (4 %) .النتائج تؤكد بان نظام الجرعة الواحدة كان اكثر اماناً من خلال تقليل الحالات الجديدة من التلف في الاذن والكلى .

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Introduction

Clinical experience over the past 50 years has shown that multiple daily dosing strategy to be both labor and lab. intensive and the Correct multiple daily dosing of aminoglycoside often requires pharmacokinetics expertise, close monitoring of drug serum levels and renal function [(1)].Currently many centers are adopting the SDD regimen as the standard / preferred dosing method and by the year 2000, about 80% of the hospitals worldwide use SDD regimen. [(2)]. The rationales of using aminoglycosides as SDD were due to their concentration dependent killing [(3)],Significant Post – Antibiotic Effect (PAE) [(4)], avoidance of the adaptive post – exposure resistance [(5)] ,and aminoglycosides' uptake into renal tubule cells and inner ear is a saturable process [(6)].The toxicities of aminoglycosides include nephrotoxicity, ototoxicity (vestibular and auditory). Approximately 8% to 26% of patients who receive aminoglycoside for more than several days develop mild renal impairment which is reversible because the proximal tubular cells have the capacity to regenerate [(7)].Aminoglycosides are poly cationic in nature binding to the anionic site on the endothelial cells of the glomerulus leading to reduction in the glomerular filtration rate.[(8)]They are almost exclusively filtered by the glomerulus and excreted unchanged. Filtered aminoglycosides undergo proximal tubular reabsorption by binding to anionic phospholipids in the brush boarder, followed by endocytosis and sequestration in lysosomes of the S1andS2 segments of the proximal tubule .[(9)] The earliest lesion observed following clinically relevant doses of aminoglycosides is an increase in the size and number of lysosomes.[(10)].These lysosomes contain myeloid bodies, which are electron-dense lamellar structures containing undergrated phospholipids .The renal phospholipidosis produced by the aminoglycosides is thought to occur through their inhibition of lysosomal hydrolyases,such as sphingomyelinase and phospholipases [(11)],as a result the lysosomes become progressively distended until they rupture, releasing lysosomal enzymes and high concentration of aminoglycosides into the cytoplasm .The released lysosomal contents can interact with various membranes and organelles that trigger cell death [(12)]. Aminoglycosides also inhibit various ATPase including Na^{+}-K^{+} ATPase, adenylate cyclase; alter the function of mitochondria and ribosome. [(13)] Aminoglycosides induce irreversible ototoxicity (Vestibular and auditory) in about 2 to 25% of the patients[(14)]. The precise mechanism of hair cell destruction in both forms of ototoxicity is unclear,but it has been suggested that aminoglycosides interfere with active transport system essential for the maintenance of the ionic balance of the endolymph [(15)]. This would lead to alteration in the normal concentration of ions in the labyrinthine fluid with impairment of electronic activity and nerve conduction .Eventually, the electrolyte changes, or perhaps the drugs themselves damage the hair cell irreversibly. Several factors have been associated with a higher incidence of ototoxicity including duration of therapy (>8 days), cumulative dose, total daily dose, trough serum drug concentration, concurrent diuretic therapy, underlying disease state, previous exposure to aminoglycoside therapy , age, specific aminoglycosides [(16)]. SDD regimen was suitable for all patients requiring aminoglycoside therapy except those having great changes in aminoglycoside pharmacokinetics like pregnant patients, children etc.....The drug dosage in SDD regimen was by fixing the dose of the drug with changing the interval of drug administration according to the estimated CLcr [(6)] and this consist of giving a constant dose of 5mg/kg/day for gentamicin and tobramycin, 20mg /kg/day for amikacin and 15 mg/kg/day for streptomycin [(17)] . The interval of drug administration will depend on the estimated CLcr calculated by using Cockcroft equation [(17)] .The dose is calculated based on Actual Body Weight (ABW) unless the patient is ≥20 % more than the IBW .For obese patients, a dosing weight (DW) should be calculated. Monitoring of renal toxicity was done through measurement of basal and every 2-3 days of serum creatinine concentration (S.cr) [(6)] .The standard measurement of peak and trough level is not required due to the high peak level and drug –free interval obtained with SDD regimen. In patients with adequate renal function (CLcr >60ml/min), the trough aminoglycoside level would be near zero (<< 1mg/L) so, the initial dosing interval would be maintained and no further drug level determination was necessary as long as CLcr remains unchanged [(18)]. In general, monitoring of the SDD regimen can be achieved by taking a single random blood sample through 6-14 hours. after starting of the infusion and the level will be evaluated on a nomogram .Even this single random drug concentration may no longer be necessary to be measured for the following classes of patients [(17)] :-Patients receiving SDD every 24 hours, Patients without concurrently administered nephrotoxic

agents ,Patients without exposure to contrast media, Patients not in the intensive care unit, Patients less than 60 years age.
A baseline and weekly audiometry are recommended for patients who require greater than 2 weeks of therapy [7].

Materials and Methods

This comparative study was done in Al-Kirkuk General Hospital in Kirkuk city on (75) patients admitted to surgery, medicine and gynecology wards under medical supervision by the specialist physician in each ward. The patients were selected randomly and they were classified in to two groups, SDD group (50 patients, age 17-79 years) and MDD group (25 patients, age 13-70 years). The Patients that were eligible for the study include all the patients requiring aminoglycoside therapy except those with great change in AG pharmacokinetics. The Patients in SDD group were received aminoglycoside antibiotic (whether gentamicin or amikacin) in a dose depending on their Actual body weight (ABW) unless their ABW is >20% above their Ideal body weight (IBW) .For these patients, a dosing weight (DW) , which is based on the IBW plus 40% of the estimated adipose tissue mass, was calculated for dosage determination. The interval of drug administration is based on the calculated creatinine clearance (CLcr) according the Cockroft Equation, which is equal to:-
CLcr (male) =IBW (kg) (140-Age) /72[S.cr mg/dl]
CLcr (female) = 0.85 IBW (kg) (140- Age) /72 [S.cr mg/dl]
Where: - IBW is ideal body weight.
Therefore,
If CLcr is ≥ 60 ml/ min, the interval of drug administration would be 24 hours,
CLcr is 59-40 ml/ min; the interval of drug administration would be 36 hours,
CLcr is 39-30 ml / min; the interval of drug administration would be 48 hours,
CLcr is < 30 ml/ min, aminoglycosides would not be recommended and other alternative antibiotics should be used.

Culture and sensitivity test was done before starting therapy using modified Kirby-Bauer Method to know the reason of failure (if occur) whether due to drug resistance or due to the regimen itself. The following laboratory tests and parameters were monitored for all patients who enrolled in the study including:- Basal and every 2-3 days measurement of serum creatinine concentration, White Blood Cell count(WBC) and general urine examination (G.U.E)(For those having UTI) , Basal and daily recording of body temperature.Monitoring of nephrotoxicity was done through basal and periodic measurement of S.cr, while ototoxicity was monitored through baseline audiometry and documentation of auditory function, and daily monitoring of any changes in hearing status under the supervision of specialized physician.

Results and Discussion

Age was considered an important factor in aminoglycosides toxicities since with increasing age there was a decrease in renal function and subsequent reduction in aminoglycosides excretion and hence there accumulation. Table(1) showed Percent of patients in respect to their age distribution.

Table (1): Percent of patients in respect to their age distribution.

Group	≤40 years	41-50 years	51-60 years	61-70 years	>70 years
SDD	40%	18%	26%	12%	4%
MDD	52%	20%	20%	8%	0%

Figure (1) showed that in the SDD group, majority (58%) of patients received the drug(gentamicin and amikacin) every 24 hours.The 36- and -48 hours intervals were used for 34 and 8% of the population, respectively. While all the patients in MDD group were received therapy every 8 hours, Renal function is an important criteria in aminoglycosides therapy since it determines the dose and interval of drug administration because they are mainly renally excreted [19].Renal function status was reflected by normal serum [S.Cr] and creatinine clearance [20]. The normal range of serum creatinine was 0.7-1.2 mg/dl (70-150 µmol/L) and of creatinine clearance was 60-125ml/min [21].

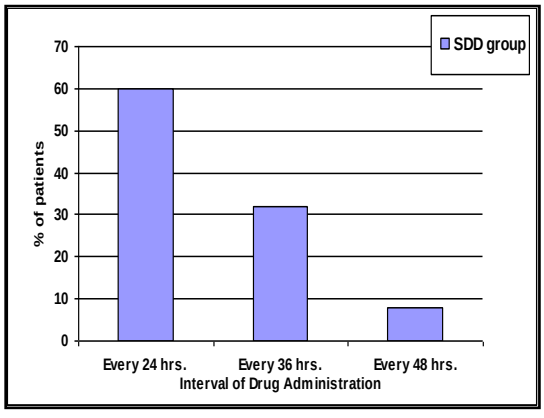


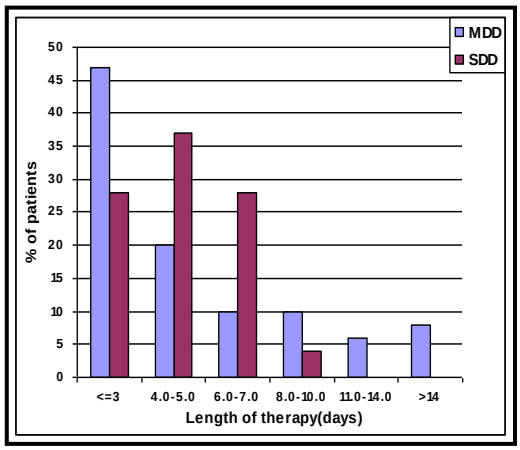
Figure (1) : Percent of patients (SDD) group in respect of intreval of drug administration

Table(2): The mean pre and post treatment serum creatinine concentration [S.Cr] for SDD and MDD group.

Groups	Pretreatment mean [S.Cr](mg/dl)	Post treatment mean serum [S.Cr] (mg/dl)
SDDgroup	1.0±0.09[a]	0.97±0.09[a]
MDD group	1.15±0.32[b]	1.7±0.33[c]

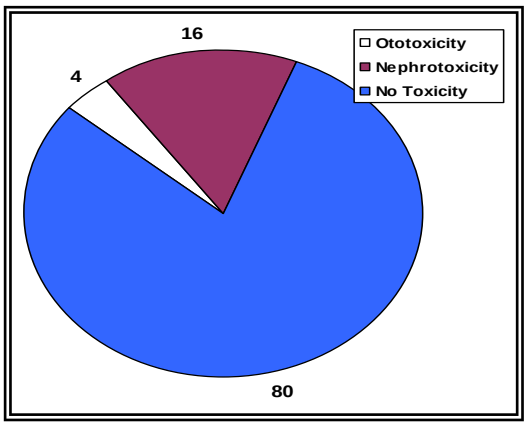
Each value represents mean±SEM, Values with non identical superscripts (a,b,c) are considered significantly different (p<0.01) .

Table (2) showed that the pre treatment renal function in both SDD and MDD group was normal (reflected by the normal [S.Cr]) while the post treatment was abnormal in MDD group (reflected by the elevated [S.Cr]) which indicate the occurrence of nephrotoxicity.Statistical analysis (P>0.01) revealed that there was no significant difference between the mean pre and post treatment [S.Cr] in SDD group while there was a significant difference (P<0.01) in MDD group. This indicates the superiority of SDD regimen over MDD regimen since the incidence of nephrotoxicity with MDD regimen was higher and occur in a wide number of patients (5-25%) as many studies reported [22] Duration of therapy (>8 days) was one of the most important determinant risk factor in aminoglycosides toxicity [23] and they receive therapy for various duration ranging from ≤3 days to > 14 days, figure (2).



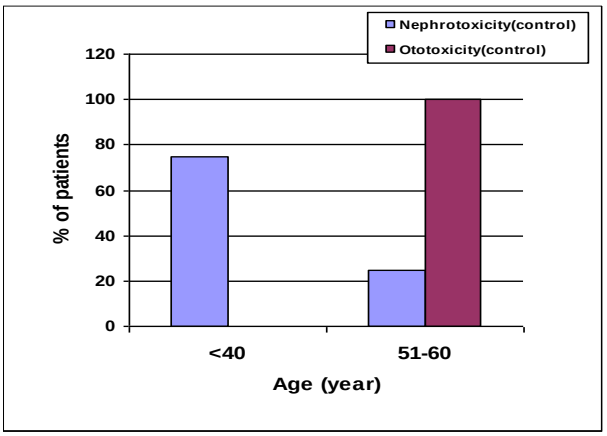
Figure(2) : Percent of patient in respect to their length of therapy

The mean length of therapy in SDD group was 6.4±1.73 days while 5.0±0.91 days in MDD group. The results of statistical analysis indicated that there was no significant difference (P>0.01) in the mean length of therapy between the SDD and MDD group.24% of the patients in SDD group received therapy for ≥ 8 days compared to only 8% in group indicating the safety of SDD regimen since no toxicity was observed in spite of higher percent of patients who received longer duration of therapy due to the safety profile of SDD regimen [24] figure (2) . Ninety six percent of the patients in SDD group received gentamicin and 24% received amikacin, while all the patients in the MDD group received gentamicin only. The total number of the patients who received gentamicin and amikacin in SDD group were > 50 because most of the patients were first treated empirically with gentamicin then converted to amikacin according to the results of culture and sensitivity test which revealed gentamicin resistant bacteria and amikacin sensitive bacteria. The mean dose of gentamicin was 282.9±6.8 {range, 212.5 - 438.4} mg in SDD group and 235.2±4.8 {range, 120- 240} mg in MDD group, while for amikacin it was1182±71.3 . {range, 914-1680} mg. The results of statistical analysis showed that their was a significant difference (P<0.01) in the mean dose of gentamicin between SDD and MDD group. This proves the safety of SDD regimen because no incidence of toxicity despite the use of higher dose, in contrast to development of toxicity in MDD group [25]. All patients who are given the SDD and MDD regimen either had improved or complete resolution of their infections except two patients given MDD regimen had failures. Concerning the toxicity (nephrotoxicity and ototoxicity) of both SDD and MDD regimen, no toxicity was observed in any patients received SDD regimen While 20% of the patients who received MDD regimen developed toxicity, figure(3).



Figure(3) : Percent of patients MDD group in respect of drug toxicity occurance

Nephrotoxicity was defined as an increase in S.cr. of ≥0.5 mg/dL above the baseline value. Patients were not evaluated for nephrotoxicity if they had been treated with aminoglycoside in the previous week, treatment with aminoglycoside was resumed within one week of stopping treatment, hemodailysis was started within 48 hour after the start of therapy and if the patient met the criteria for nephrotoxicity within the first 24 hour of therapy since in that case the decline in renal function is unlikely to be the result of the aminoglycoside treatment [(26)]. Nephrotoxicity was not detected in any of the patients who received SDD regimen. This agree with other study which also indicate no nephrotoxicity with SDD regimen[(27)] , but David P. Nicolau study showed that SDD regimen may be associated with small percent (1.2%) of nephrotoxicity [(6)] . Sixten percent of the patients in MDD group met the criteria of nephrotoxicity in our study, figure (3). Figure (4) show the age distribution of the patients in whom toxicity occurred. All the patients who developed nephrotoxicity in MDD group were male and 75% were of age of ≤ 40 years with mean age of 35±9.5 years and mean dose 210±20.0 mg. This agree with other studies that revealed 5-25% of nephrotoxicity with MDD regimen [(28,29)].The mean length of therapy in those who developed nephrotoxicity was 9.3±2.9 since duration of therapy was of the most important determinant factor in nephrotoxicity [(30)],

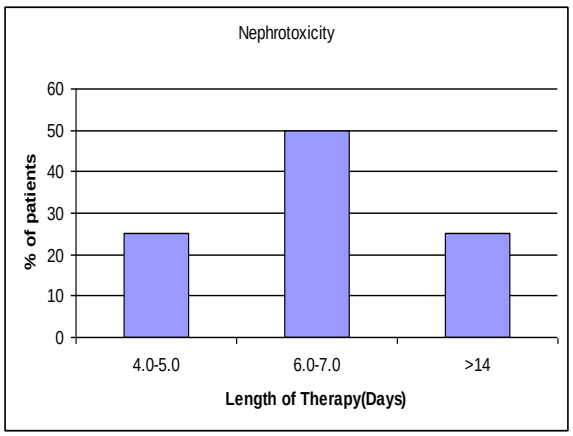


Figure(4) : Percent of patient MDD group in whom toxicity occur in respect to age distribution

Figure(5) . No significant difference in the mean age and dose was observed between the patients in MDD group who developed nephrotoxicity and those who did not, but there was a significant difference ((P<0.01) in the mean length of therapy, 5 days compared to 9.3 days, indicating the importance of duration of therapy on toxicity. Ototoxicity, in the form of vestibular manifestation, was not observed in any of the patients in SDD group as in Benjman M. Limson *et al* study which also revealed no ototoxicity with SDD regimen [(31)] while other study revealed only 0.14% ototoxicity[(6)]. Four percent of the patients in MDD group developed ototoxicity with age >50 years old, and duration of therapy of >14 days as Bates, D.E. study indicate a high percent of ototoxicity with MDD regimen ranging from 5-25% [(32)] .

In spite of the presence of greater risk factors for aminoglycosides toxicity in SDD group including :-

- elderly patients(>70 years) were found in SDD group but not in MDD group;
- the percentage of patients who received therapy for ≥8 days were greater in SDD group;
- administration of amikacin in SDD group for a median length of therapy of 13 days(range 3 to 42 days) which is greater than the recommended duration of therapy for amikacin(i.e., 7 to 10 days);
- greater median dose of gentamicin in SDD group;
- use of SDD regimen in diabetic and hypertensive patients with impaired renal function;



Figure(5) : Percent of patients MDD group in whom nephrotoxicity occur in respect of length of therapy

with no appearance of toxicity in SDD regimen indicates it's safety over MDD regimen. This safety was due to the new pharmacodynamic data of aminoglycosides which is the saturability of aminoglycosides accumulation in renal tubular cells and inner

ear at low concentration (threshold concentration) and the time above this threshold concentration was the determinant of toxicity[6, 33]. The threshold for toxicity was corresponds to a plasma concentration of 2 mg/L. The once-daily regimen produces a threefold higher plasma concentration, which enhance efficacy that otherwise might be compromised due to the prolonged sub-MIC concentration later in the dosing interval compared with every-8-hour regimen. Once-daily regimen provides a 12-hour period during which plasma concentration are below the threshold for toxicity, thereby minimizing the toxicity that otherwise might result from the early high plasma concentration. The every-8-hour regimen, in contrast, provides only a brief period (< 3 hours) during which plasma concentrations are below the threshold for toxicity [33,34] , (figure 5).

Conclusion

On the basis of the obtained results, one can conclude that SDD regimen appears to be safer than the conventional MDD regimen through reduction in the incidence of nephrotoxicity and ototoxicity.

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Isolation of Some Microorganisms from Bar Soaps and Liquid Soaps in Hospital Environments

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Abstract

This study was designed to determine the colonization of the in-use hand washing soaps in hospital settings. It is a comparative cross-sectional research in a surgical specialties and Baghdad teaching hospital in Baghdad, Iraq. Swabs from surfaces of bar soaps and from liquid soaps via their applicator tips; at the sinks of toilets of hospital staff and working rooms of the wards were taken in January 2008. Conventional microbiologic methods were used for culture of the swabs and identification of the isolates. Colonization was detected 60% and 15.9% in bars and liquid forms respectively. And this lead to the conclusion that bar soaps could be colonized with microorganisms excessively. Liquid hand washing soaps are more appropriate in hospital environments. Proper using conditions of the hand washing items should be defined in health care settings.

Keywords: Bar soap, liquid soap, *pseudomonas aeruginosa*, nosocomial infections.

الخلاصة

تلوث اليد بالبكتريا يعتبر من أهم الطرق لانتقال العدوى بين المرضى أو من العاملين في مجال الرعاية الصحية إلى المريض. نظافة اليد تعتبر أهم أداة في السيطرة على حالات العدوى المستشفويه. هدفت هذه الدراسة لتحديد بعض الإحياء المجهرية المستعمرة على أنواع الصابون المستخدم في المستشفيات. صممت هذه الدراسة على شكل مقارنة مقطعية وتم جمع مسحات من أسطح الصابون الصلب ومن فتحات جهاز الإعطاء للصابون السائل في مستشفى بغداد التعليمي و الجراحات التخصصية في بغداد, العراق. أظهرت النتائج أن نسبة تلوث الصابون الصلب كانت 60% بينما كانت 15.9% للصابون السائل. هذه النتائج تؤكد أن استعمال الصابون السائل بالمستشفيات انسب من الصابون الصلب.

Introduction

Hand carriage of bacteria is an important route of transmission of infection between patients or from the health care worker to the patient.[1-6] Hand hygiene has been considered to be the most important tool in nosocomial infections control. Failure to perform appropriate hand hygiene is supposed to be the leading cause of nosocomial infections and the spread of multiresistant microorganisms, and has been recognized as a significant contributor to outbreaks. The microbial flora of the skin of hands consists of resident and transient microorganisms. The resident microorganisms survive and multiply on the skin. The transient microorganisms represent recent contaminants of the hands acquired from colonized or infected patients/clients or contaminated environment or equipment. Transient microorganisms are not consistently isolated from most persons. In contrast to the resident microorganisms, the transient microorganisms found on the hands of health care personnel are more frequently implicated as the source of nosocomial infections. The most common transient microorganisms include gram negative coliforms and *Staphylococcus aureus.* Hand washing with plain soap is effective in removing most transient microrganisms.[7-9] The mechanical action of washing and rinsing removes most of the transient microorganism present.[10-12].Health care workers wash their hands in two ways: (a) the social hand wash, which is the cleaning of hands with plain, non-medicated bar or liquid soap and water for removal of dirt, soil, and various organic substances; (b) the hygienic or antiseptic hand wash, which is the cleaning of hands with antimicrobial or medicated soap and water. Most antimicrobial soaps contain a single active agent and are usually available as liquid preparations. Appropriate hand washing results in a reduced incidence of both nosocomial and community infections.[13] Much studies have been written and debated regarding the use of bar versus liquid skin cleansers in relation to infection control.[7,14-22] In this study, the aim was to detect and compare bacterial contamination of soap bars and liquid soaps.

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Materials and Methods

Setting

Surgical specialties and Baghdad teaching hospital in Baghdad, at the middle of Iraq.

Materials

In January 2008, 50 swabs from surfaces of bar soaps at the sinks of toilets and working rooms of the wards were collected; 44 swabs were collected from tips of the applicators of liquid soaps containers approximately at the same hospital points. Swabs were collected from wet surfaces of bars and tip of the containers of liquid soaps. Soap bars were plain soaps (Duru, Turkey). Liquid soaps (Johnson, Turkey) included formaldehyde (in trace amount as antiseptic agent according to label information). Despite of the liquid soaps in this settings were called antibacterial by the manufacturer; formaldehyde that was included in the liquid soaps considered as preservative rather than antibacterial effect.

Microbiology

Collected swabs were dipped into tubes containing 1 ml sterile normal saline (0.9%). Samples were brought to the microbiology laboratory without delay. Tubes were shaked and Ten microliter of substance was inoculated on blood agar and eosin-methylen-blue (EMB) agar incubated at 37 ^{0}C for 22-24h. Sabouraud`s agar media are enforced with chloramphenicol (16 µg/mL) to inhibit the growth of contaminating bacteria; incubated at 30C$^{\circ}$, for 30 days were used to rule out fungi . Unfortunately anaerobic laboratory conditions could not be accomplished during this study due to the shortage in laboratory facilities. Yielded microorganisms were identified by conventional microbiological methods and by using API 20E, API 20NE & API Strep (Biomerieux, USA).

Statistics

T-tests were used for calculating significance of difference of colonization rates between bar soaps and liquid soaps and also comparing the frequency of yielding microorganisms.

Results

Among 50 swabs of bar soaps, 30 (60%) swabs were found colonized. A total of 44 microorganisms were isolated. Numbers of isolate are shown in figure 1. *Pseudomonas aeruginosa* (41%) was the most frequent isolated bacteria followed by *Escherichia coli* (13.6%) and *Acinetobacter baumanii* (11.4%).From liquid soaps, 6 microorganisms were detected at only 7 tips (15.9%) of the total 44 containers. This includes 4 (66.6%) *P. aeruginosa*, one (16.6%) *Proteus penneri* and one (16.6%) *Flavimonas oryzihabitans.*

Comparison of the rates of bacterial colonization between bar soaps and liquid soaps are shown in figure 2 and 3.Bar soaps were found more colonized than the liquid soaps significantly ($p<0.05$). *P. aeruginosa* was the most frequent isolate in both two group whereas isolation rate was significantly higher ($p<0.05$) in bar soaps but not in the liquid soaps ($p>0.05$) as statistically.

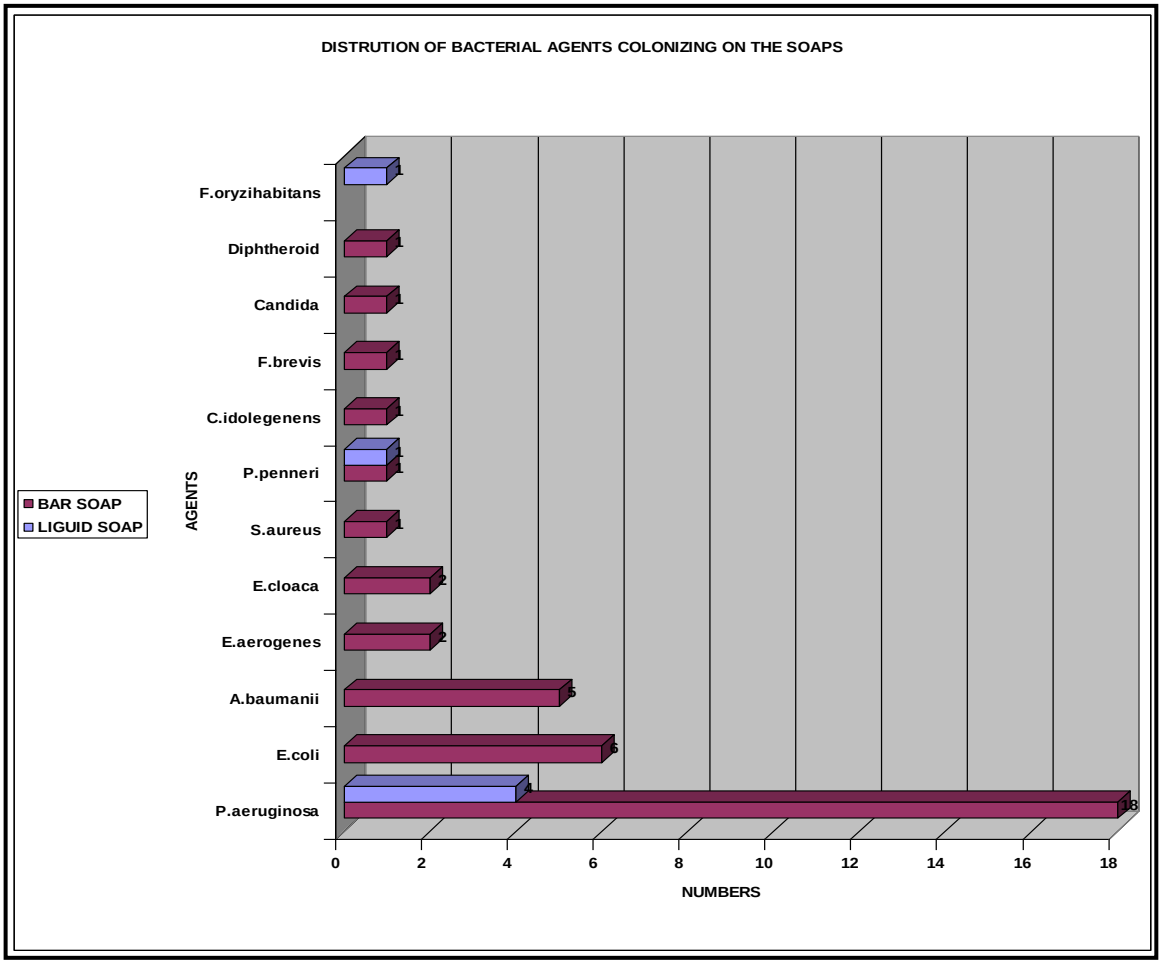

Figure 1. Distribution of bacterial agents colonizing on the soaps

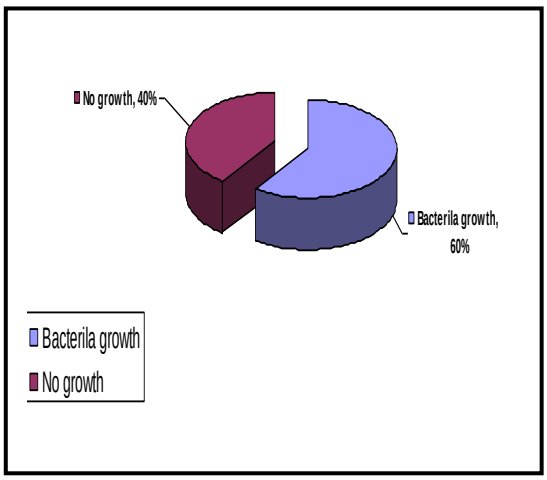

Figure 2. Bacterial colonization rates of bar soaps

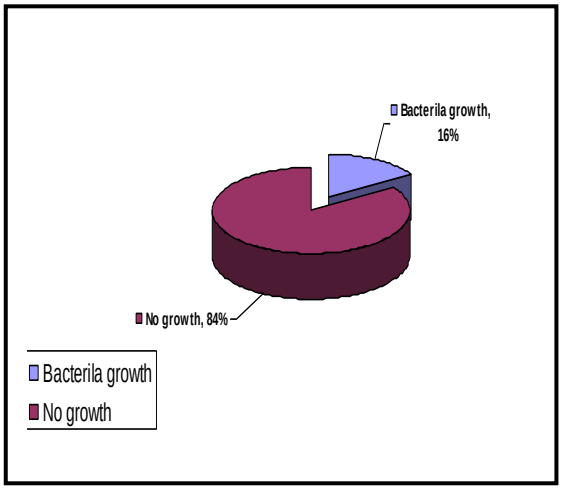

Figure 3. Bacterial colonization rates of liquid soaps

Discussion

The most common hand-cleaning agents are bar soap and liquid soap in disposable plastic containers. When in use, bar soaps are frequently misused because they are typically stored in contact with moisture and remain moist for long periods of time. It is usually kept in a container, on or next to a wash basin. More often than not, it resides in surface water. The resulting jelly mass is unsightly, difficult to use effectively. This supplies an environment which provides the perfect opportunity for bacteria and organisms to grow. Most bars of soap in communal areas are used by a number of different people. This means that one bar of soap can be in direct contact with skin bacteria from more than one person, and may harbour live pathogenic bacteria.[22] Cross infection can and does occur under these circumstances.[23] When using a bar of soap, the CDC (Centre for Disease Control) recommends placement on a drainable rack between uses.[6] Soap racks that promote drainage of all water from the bar should be installed. In addition, there should be easy access to replacements when soap is lost, dropped, melted, or consumed. Small soap bars were also recommended that can be changed and used in preference to larger bars that are more likely to melt or become colonized with bacteria.[24] Liquid soap on the other hand is much better to use. Liquid soap is dispensed straight from a plastic container. It has not been exposed to skin bacteria or other contaminants. As a result, cross contamination is not likely to occur, providing a more cleaning and more hygienic alternative.[23]McBride et al reported that bar soaps were found to have higher bacterial cultures after use than liquid soaps.[23] In another study, Kabara and Brady obtained samples from bar and liquid soaps from 26 public bathrooms which were investigated. Liquid soaps were found to be negative for bacteria, while 100% of the 84 samples obtained from bar soaps yielded positive cultures.[15] In an epidemiological study, the researchers isolated several strains of *Pseudomonas* from 45 of 353 environmental samples used by multiple providers (13%) and found that the 5 most common strains were frequently found on patients. They also affirmed that the hands are a major vehicle for the transfer of *Pseudomonas* bacteria and implicated bar soap in its spread.[14] Other groups of researchers have found that bacteria survive on soap bars in continuous use in public lavatories, even when cultured 48 hours following their last use.[15,22] The role of the soap dish in infection control has also been studied. Despite of CDC recommendations most health care settings like our hospital are using soap dishes instead of drainable racks. Jarvis et al showed that supplies used for hand washing can be contaminated with gram-negative organisms if they are not completely dried. Swabs were collected from soap dishes on 6 wards and from a bacteriology laboratory on 4 consecutive days. The sludge of the dish was found to be colonized with predominantly gram-negative bacteria. This colonization persisted, even when medicated iodophor bar soap was used.[25] In our study, dishes were found wet, and surfaces of soaps were generally covered by squashy mass and bars were found heavily contaminated (%88). This study revealed quite lower contamination rate in liquid soaps compared with bar soaps, although they didn't include suggested antibacterial agents for hand antisepsis such as triclorasan or chlorhexidine. However, liquid soaps would be expected to be sterile. So, there should be problems with the handling. Honestly, in this study any strict procedures had not been followed in the wards for the how often liquid dispensers should be cleaned, disinfected or exchanged. After the results were obtained, procedures were described for handling and usage of liquid soaps and dispensers immediately. In conclusion, correct use of hand washing materials is more important choosing kind of soaps. Hypothesis of transferring microorganisms to healthcare workers' hands via contaminated soap bars have not confirmed, antibacterial or not, liquid soaps seem more suitable alternative for hygienic hand washing. Proper handling of liquid soap should be implemented wherever they are used in the hospital. Compliance of the hand washing is more important than the kind of the soap.

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The Inhibitory Effect of Gallic Acid on Human Serum Cholinesterase

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Abstract

The dried fruit peel of pomegranate in *Punicaceae* family was fractionated chromatographically on Sephadex-LH-20 column .Gallic acid (trihydroxybenzoic acid) and its related galloyl esters such as gallotannin(i.e. β-penta-O-galloyl–D-glucose) were obtained homogenously. Different concentrations of gallic acid and gallotannin were used to determine their inhibitory effect on human serum cholinesterase. The enzyme activity was measured according to the method reported by the WHO .The inhibitory effect of these compounds on the activity of human serum cholinesterase have been studied in vitro .The inhibitory effect was remarkably clear with increasing concentration of gallic acid .Whereas galloyl ester showed no inhibitory effect. The inhibition with gallic acid indicates a noncompetitive pattern. Therefore, we can not recommended gallic acid and its related compounds , as preservative substances in food industry or in pharmacological preparations since they might have some side effect on certain biological systems..

Key words*:* Gallic acid , gallotannin , Human Serum Cholinesterase .

الخلاصة

تم فصل بعض المركبات من القشور الجافة لثمرة الرمان من العائلة الرمانية(*Punicaceae family*) بواسطة العمود السائل اللوني الحاوي على مادة السفادكس ألـ20.و تم الحصول على مادتين متجانستين هما حامض الكاليك (trihydroxybenzoic benzoic acid) وأحد مركبات أسترات الكالوليل التابعة له وهي مادة الكالوتانين(β-penta –O-galloyl –D-glucose) . أن تأثير حامض الكاليك و الكالوتانين لم تدرس بشكل واسع على فعالية ألأنزيمات. استخدمت تراكيز مختلفة من حامض الكاليك والكالوتابين لقياس تأثير تثبيطهما على مصل الكوليستريز(cholinesterase) البشري . أن فعالية هذا الإنزيم تم قياسها طبقا لطريقة منظمة الصحة العالمية (WHO) .. هذه الدراسة اختيرت لتقييم فعالية هذه المركبات مختبريا على مصل الكوليستريز البشري لزيادة معرفتنا حول الفعاليات البيولوجية . أن تأثير تثبيط هذه المركبات جدير بالملاحظة مع زيادة بتراكيز حامض الكاليك بينما لم يلاحظ أي تثبيط معنوي لمادة الكالوتانين . وأن التثبيط بحامض الكاليك تكشف بأنه غير تنافسي بطبيعته .لذلك لا نستطيع أن نوصى بحامض الكاليك والمركبات التابعة له كمواد حافظة في الصناعات الغذائية أو في التحضيرات الصيدلانية نظرا" لاحتمال احتوائها على بعض التأثيرات الجانبية على بعض الأنظمة البيولوجية .

Introduction

Phenolic compounds are secondary plant products which rarely occur in the free state in living plant tissue. Simple phenols are caustic substances and well known to be anti-microbial agents[(1-3)]. Polyphenols like lignin and tannins are also found in plant cells. Tannins or tannic acids are believed to be the most important group of secondary metabolites involved in plant defense[(4-6)] . It has been found that tannins have shown potential antiviral[(7,8)], antibacterial [(9,10)] and antiparasitic effects [(11)]. In the past few years tannins have also been studied for their potential effects against cancer through different mechanisms [(12-14)] . Tannic acids are not single homogeneous compounds, but a mixture of esters of gallic acids with glucose whose exact composition varies according to their sources[(15)] . The biological activation of gallic acid and its related galloyl esters have not yet been studied widely regarding their effect on enzymes. They are employed in medicine as astringents in the gastrointestinal tract (GIT) and on skin abrasions. In the treatment of burns, the proteins of the exposed tissues are precipitated to form a mildly antiseptic, protective coat under which the regeneration of new tissues may takes place[(16)]. Many plant species native to Iraq are known to contain certain chemical compounds which exert their effects on different biological system within the cellular level such as enzymes[(17-19)]. Chemically, these complex substances are usually occur as a mixture of polyphenols that are difficult to separate because they do not crystallize , the application of some chromatographic methods has enabled to confirm the complicated nature of these polyphenolic extracts and also to identify the simple phenols present in small amounts in such mixtures[(20,21)]. In addition, it is of interest to improve methods of separation and identification of gallic acid and some of its related esters obtained from Iraqi plants . Aim of this work was conducted to study the effect of gallic acid and its related glucose esters such as gallotannins on human serum cholinesterase in vitro.

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Materials and Methods

Extraction of plant material:

The fruit peel of Punicaceae in pomegranate family were obtained from Iraqi market. The peels of healthy fruit were dried at room temperature for at least six months, before they were powdered in a mortar and the powder was sieved through 100-150 mesh sieve.The powder (50g) was boiled for few minutes in 100ml ethanol (95%) and left with stirring for at least 3hr. The extract was decanted through fiber glass. The remaining residue was re-extracted twice with ethanol and the combined extracts were concentrated in vacuo to remove ethanol, the heavy viscous residue obtained as the phenolics materials[20,21].

Isolation and identification of phenolic substances:

Phenolic materials were chromatographed on Whatman No.1 paper in two dimensions with 6% (V/V) acetic acid (Solvent A) and isobutanol-acetic acid-water (14:1:5) (Solvent B)at 25±1°C . Phenolic compounds such as gallic acid and gallotannin (Tabel-1)were revealed by spraying with a freshly prepared reagents[15,21-23,] of ferric chloride-potassium Ferricyanide ,Gibbs reagent ,Saturated aqueous potassium iodate(KIO_3) reagent , and finally a fresh solution of nitrous acid reagent was prepared to give the phenolic compounds a characteristic color which helps in their identification . The phenolic extracts (10g) obtained as mentioned above was fractionated chromatographically on Sephadex LH-20 column (100x2.5cm) using the same methods as described previously[21,24]. Sephadex LH-20 is very useful for separation tannin from nontannic phenols[25] .Table-2 showed two substances (i.e. gallic acid and gallotannin) were obtained homogenously by fractionation .

The resultant substances ,as in the following :

1-Gallic acid : Fractions 2 (130 ml) was dried at 25°C and 0.01mmHg over phosphorous pentoxide and rechromatographed once again over Sephadex-LH-20 column . The dried substance gave a pale-yellow-white form melting point (m.p.) 250-253°C. Rf values, showed 0.52 and 0.6 with solvent A and B respectively .Table-1 shows paper chromatograms when treated with a freshly prepared reagents revealed a characteristic colors exhibited by this compound. So under short -u.v. light (254nm) the chromatogram. gave soft blue- violet appearance in visible light which turned to deep violet upon exposure to fuming ammonia. Elemental analysis found ; C, 44.73% ,H, 4.34 %, calc. for $C_7H_6O_5$, as amorphous compound ,(Lit[25] C , 44.70 % ,H,4.31%).

2- Gallotannin;.:Fraction 6 (105 ml) was dried and then rechromatographed in the same procedure as did for gallic acid . The dried substance gave an off-white granular solid; m.p. 200-210°C. Rf values on paper chromatogram showed 0.06 and 0.5 with solvents A and B, respectively.Table-1 shows a characteristic colors exhibited by glallotannin on paper chromatograms when sprayed with reagents .Gallotannin normally showed up as brown-purple spot on chromatograms when treated with Gibbs reagent. The chromatogram also showed a pink appearance with ferric chloride-potassium ferricyanide in visible light which turned to dark- blue absorption in u.v. light which enhanced by fuming with ammonia .Specific spray for gallotannins is Potassium iodate solution, which gives a rose –pink color and reacts with gallic acids to form the characteristic orange of purpurogallin carboxylic acids[26,27]..Elemental analysis showed; C, 52.38 % ; H , 3.48 %; calc for $C_{41}H_{32}O_{26}$, as amorphous compound , (Lit[25].C,52.35 %,H, 3.50 %) .

Table(1) : Detection of gallic acid and gallotannin by various sprays .

Compound	Spray				u.v light
	Ferric Chloride-Potassium ferricyanide	Gibbs reagent	potassium iodate	nitrous acid	
Gallic acid	Blue	Brown	Orange→red→pink	Brown	Blue-violet→deep ep violet
gallotannin	Pink	Brown-purpule	Rose →pink→orange	Brown	Dark blue

Enzyme activity determination:

Different concentrations (ranging from 6mM/L to 30mM/L)of gallic acid and gallotannin were used to determine their inhibitory effect on human serum cholinesterase in vitro. The enzyme activity was measured according to the method reported by the WHO[28], with minor modification as described in previous study [17]. Enzyme activity was expressed as µ moles of substrate (acetylthiocholine iodide) hydrolyzed per ml of total mixture per min.

Results and Discussion

Chromatographic results for the fractions of the extract to detect the phenolic substances as compared with authentic compounds (Tables-1 and- 2) showed that fraction 2 was identified as gallic acid, whereas fraction 6 identified as gallotannin .Also the u.v. light detection ,various sprays, melting points and elemental analysis were applied to identify these compounds . These findings agree with standard authentic compounds were obtained from pharmacy college stores (i.e. gallic acids and gallotannin) which showed in such close agreement as to indicate the identity of the substance previously .Also these finding agree with earlier reports [15,20 ,25,27] No clear inhibitory effect could be detected with different concentrations of gallotannin (ranging from 6 mM/ L to 35mM/l) on human serum cholinesterase. This might be attributed to the presence of amber color which may interfere with the color developed as a result of enzymatic reaction. Decolorization of this solution might merit different results.The inhibitory effects of different concentrations of gallic acid on the enzyme activity were summarized in Table 3 .It was found that increasing gallic acid concentrations will accordingly affect enzyme activity. Gallic acid concentration as low as 6mM/L results in approximately 10% inhibition ($p<0.001$) and reaching to about 50% inhibition with increasing concentration as high as 30 mM/L ($p<0.0001$). Such highly significant inhibition was unexpected firstly, because gallic acid which was considered as one of the phenolic compounds used as antimicrobial agent in industry[20,29], and secondly, it has not been reported before that phenolic compounds derived from native Iraqi plants exert such inhibitory effect on this enzymatic system i.e. human serum cholinesterase , a well-known biological function of being a neurotransmitter in animals and insects[30] , its has a very high catalytic activity , that catalyzes the hydrolysis of the neurotransmitter acetylcholine into choline and acetic acid, a reaction necessary to allow a cholinergic neuron to return to its resting state after activation[31] .Furthermore, the reciprocal Line weaver-Burk plot for the rate of reaction versus substrate concentration in the presence and absence of gallic acid (Fig.1) showed that the inhibition follows a noncompetitive pattern. This might be explained by that the inhibitor can not bound to the anionic site in the catalytic centre of the enzyme[32]. The mechanism of action of such bounding might be explained through the hydrogen bounding between the carboxyl group of the inhibitor and some catalytically significant group of the enzyme probably with the imidazole moiety of histidine in the static site of the enzyme molecule[33] .

Table(2) : Fractionations of gallic acid and gallotannin by column chromatography using Sephadex LH-20

Fractions	Volume (ml)	Elution Solvent	Weight after drying (g)	Substance
1	212	Ethanol (100%)	5.56	Unknown
2	130	Ethanol (100%)	1.42	Gallic acid
3	137	Ethanol (90%)	1.70	Mixture of gallic acid and other phenolic substances .
4	125	Ethanol (90%)	-	
5	118	Ethanol (90%)	-	
6	105	Ethanol (70%)	0.68	gallotannin
7	195	Ethanol (60%)	0.54	Polyphenolic substances

Table(3) :In vitro inhibition of human serum cholinesterase by different concentrations of Gallic acid.

Inhibitor mM/L	Enzyme Activity U/ml	% Inhibition	Recovery %
Nil	5.93 +1.8	Nil	100.00
06	5.44+ 1.6	08.22	91.69
12	4.76+ 1.4	91.69	80.36
18	4.32+1.3	27.24	72.74
24	3.83+ 1.3	35.67	64.52
30	3.24+ 1.1	45.15	54.38

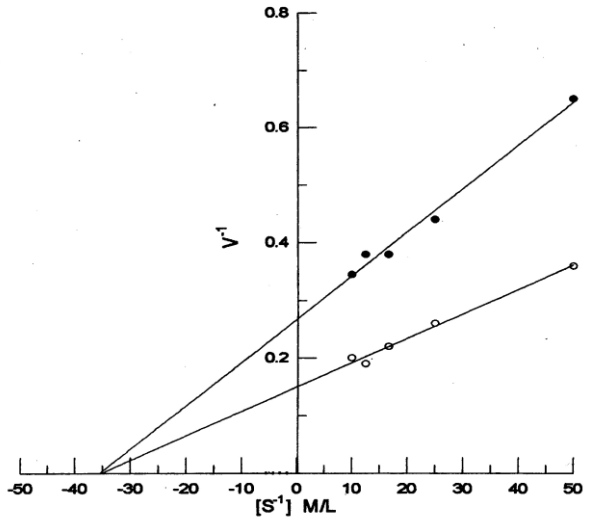

Figure (1) : Double – reciprocal plots for the inhibition of human serum cholinesterase in the presence (●) and absence of (o) of 30 mM/L gallic acid . Acetylthiocholin iodide concentrations (s) were ; 0.02, 0.04 ,0.06 ,0.08 , 0.10 M/L .

Conclusion

Phenolic compounds, such as gallic acid derived from native Iraqi plants with antimicrobial activity might not be recommended to be used as preservative in food industry or in pharmacological preparations since they might exert some undesirable effects on certain enzymatic system such as serum cholinesterase.

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Preparation of a New Dosage Form of Metoclopramide Hydrochloride as Orodispersible Tablet[#]

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Abstract

Metoclopramide HCl (MTB) is a potent antiemetic drug used for the treatment of nausea and vomiting. Many trials were made to prepare a satisfactory MTB orodispersible tablet using direct compression method.Various super disintegrants were used in this study which are croscarmellose sodium (CCS), sodium starch glycolate (SSG) and crospovidone (CP). The latter was the best in terms of showing the fastest disintegration time in the mouth.Among the different diluents utilized, it was found that a combination of microcrystalline cellulose PH101 (MCC 101), mannitol, dicalcium phosphate dihydrate (DPD) and Glycine was the best in preparing MTB orodispersible tablet with fastest disintegration time in the mouth.The physical parameters of the prepared MTB orodispersible tablet were satisfactory as hardness (4 Kg), friability (0.5%) and mouth disintegration (23 sec).The overall results suggest that the prepared formula of MTB as orodispersible tablet could be utilized as a new dosage form for the oral administration.

Key words: Orodispersible, metoclopramide, super disintegrant

الخلاصة

يعد عقار الميتوكلوبراميد هايدروكلوريد (Metoclopramide HCl) من العقارات الفعالة في علاج حالات التقيؤ. هذه الدراسة تتعلق بتصنيع الميتوكلوبراميد هايدروكلوريد على شكل حبة سريعة التحلل في الفم (orodispersible tablet) باستخدام طريقة الكبس المباشر.تم استخدام عدة مواد مفككة وهي مادة (croscarmellose sodium) ومادة (sodium starch glycolate) ومادة (crospovidone). ويعد الأخير هو الأفضل لأن وقت تحلله في الفم كان اسرع من باقي المواد المفككة. من بين مواد التخفيف المستخدمة، وجد بأن مادة السليلوز مجهري التبلور والمانيتول ومادة (dicalcium phosphate dihydrate) والكلايسين كانت هي الافضل في تصنيع حبة الميتوكلوبراميد هايدروكلوريد سريعة التحلل في الفم. وكانت الخصائص الفيزيائية لحبة الميتوكلوبراميد هايدروكلوريد سريعة التحلل في الفم جيدة فمثلا كانت صلابة الحبة ٤ كغم في حين كانت الهشاشة ٠,٥% اما وقت التحلل في الفم فهو ٢٣ ثانية.

Introduction

Orodispersible tablet is a solid dosage form containing a medicinal substance that disintegrates and/or dissolves rapidly in the mouth (either on or beneath the tongue or in the buccal cavity) without drinking water within up to three minutes. Upon placement in the mouth, orodispersible tablets absorb saliva rapidly into the tablet core allowing the super disintegrant to swell, rupture the tablet and liberate the individual components that form solution or suspension, which in turn can be swallowed easily without water. On the other hand, orodispersible tablet can also be swallowed intact as it is; i.e., as if it was a conventional tablet by using water to push it down to stomach [(1)].The orodispersible tablets have increased in popularity because consumers, all ages, find them convenient and easy to use. Since orodispersible tablets can be taken without water, therefore, bed-ridden, lying, standing, walking, talking, and traveling patients can use them easily any time and anywhere [(2)].Orodispersible tablet is the proper solution for dysphagia. Dysphagia is defined as difficulty swallowing food or liquids, which may be caused by normal physiologic response or as a result of disease state[(3)]. As a consequence of dysphagia, children and elderly patients do not take their medication as prescribed. It is estimated that as high as 50% of the population has this problem which results in a high prevalence of ineffective therapy [(4)].Other advantage of orodispersible tablets is fastening onset of action. They disintegrate in the mouth in a period of seconds in comparison to conventional tablets which may need up to 15 min to disintegrate completely in the stomach. Furthermore, a dissolution process for water-soluble drugs begins to initiate while the orodispersible tablet still in the mouth. These facts reflect why orodispersible tablets have rapid or ultra rapid onset of action [(5)].

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Many side effects are associated with the conventional dosage forms such as tablets and capsules. For example, these dosage forms may lodge esophagus, causing local irritation. When the tablet or capsule disintegrates in stomach, it may release all of its content in the same area, which may cause a gastric irritation especially if the drug is highly water soluble due to the formation of localized high concentration. Regarding orodispersible tablet, it disintegrates and/or dissolves in the mouth and diluted subsequently with saliva, so no such side effect can take place.According to the above advantages, orodispersible tablets can be used for most cases, but their benefits can be doubled in certain clinical situations; among them are nausea and vomiting. During nausea and vomiting, it is better for the patient to take orodispersible tablet and not a conventional one due to the following reasons[6]:

a. To avoid the stimulation of vomiting center in the CNS. This center is stimulated by GI distention which, in turn, is caused by food or fluid intake. If the patient takes conventional tablet, he should use a glass of water to swallow it and hence, vomiting reflux may be initiated, bringing about a new vomiting episode.

b. Physiologic defense mechanism which makes the patient reluctant to drink.

Many products of orodispersible tablets have been launched worldwide over the past decade by the most famous drug companies. AstraZeneca, GlaxoSmithKlin, Eli Lilly, Pfizer, Wyeth, Squibb, Bristol-Myers, Schering Plough, Merck, Janssen and Organon are examples on these companies [7].

Experimental Materials:

The following materials were used in this study: CCS and CP (Al-Hekma Drug Industry, Jordan), MTB, SSG and DPD (Samara Drug Industry (SDI), Iraq), lactose, mannitol and hydrochloric acid (HCl) (Riedal De Haen Ag Seelze Hanover, Germany), glycine (Fluka Chemi Ag, Switzerland), magnesium stearate (Mg St) (Barbeher, GmbH, Germany), Meclodin® tablet (SDI, Iraq), Primperan® tablet (Sanofi corp., France).

Methods:

Formulation of Orodispersible Tablet:

Formulation of Control Orodispersible Tablets (without MTB):

Different control formulas (without MTB) were prepared and tested to obtain the best formula that disintegrates as fast as possible in the mouth (table 1). All formulas were prepared using direct compression technique. Each formula was formulated by mixing all the ingredients (except the lubricant) for 15 min after which the lubricant was added and blended for another 3 min. The final mixture was compressed using a double punch, Korsch, tablet machine with a 10 mm flat punch.

Formulation of MTB Orodispersible Tablet:

MTB was incorporated with the best control formula (with regard to shortest disintegration time in the mouth) to obtain the final MTB orodispersible tablet that subjected subsequently to further investigations. The MTB orodispersible tablet was prepared by the same method mentioned above.

Physical Parameters Measurement of the Prepared Orodispersible Tablets:

Content Uniformity:

this test was undergone for the prepared MTB orodispersible tablet. The content uniformity was performed by taking ten tablets and assayed individually. The requirement for this test is met if the amount of ingredient in each of the ten tablets lies within the range of 85-115% of the label claim [8].

Hardness:

the hardness of all the prepared orodispersible tablets (with and without MTB) were measured using Monsanto hardness tester. Results are expressed as a mean ± S.D (n=3).

Friability:

the friability test was undergone for the prepared MTB orodispersible tablet using Roche friabilator for 4 min at 25 r.p.m. by taking ten tablets weighing them all together then placing them inside the tester. After their revolution, they were cleaned from dust and weighed again. The friability was calculated as the percent weight loss. If the reduction in the total mass of the tablets is more than 1%, the tablets fail the friability test [8].

Disintegration Test:

Two types of disintegration tests were done for the prepared orodispersible tablets: by using the disintegration apparatus (in vitro test) and disintegration test in the mouth using healthy volunteers (in vivo test).

Conventional Disintegration Test (in vitro test):

the disintegration time of the prepared MTB orodispersible tablet was determined in different solutions (D.W, 0.1N HCl pH 1.2 and phosphate buffer pH 6.5). In addition, the disintegration time in D.W was determined for Meclodin® and Primperan® tablets as

references. Disintegration apparatus with a ended tubes and 10-mesh screen on the bottom was used. A tablet was placed in each tube of the basket and the time for complete disintegration of the six tablets was recorded[8].

Disintegration Test in the Mouth (in vivo test)*:*

Five healthy volunteers were subjected to the measurement of the disintegration time in the mouth of all the prepared orodispersible tablets (with and without MTB). Prior to the test, all volunteers got a detailed briefing on purpose of this test, then they were asked to rinse their mouth with water. Then the prepared orodispersible tablet was placed on the tongue and immediately a stopwatch was started. They were allowed to move the orodispersible tablet against the upper palate of the mouth with their tongue and to cause a gentle tumbling action on the tablet without biting on it or tumbling it from side to side. Immediately after the last noticeable granule or fragment had disintegrated, the stopwatch was stopped and the time was recorded. The swallowing of saliva was prohibited during the test, and also saliva was rinsed from the mouth after each measurement. To check reproducibility, each volunteer repeated the test three times [9].

Dissolution Test*:*

the basket method was used to determine the release profile of the drug from the prepared MTB orodispersible tablet. The test was carried out in three different dissolution media which are 900 ml of D.W, 0.1N HCl (pH 1.2) and phosphate buffer (pH 6.5) at 37 ± 0.5 ^{0}C with constant stirring speed of 50 r.p.m for 30 min. In addition, the release profiles of basket rack assembly containing six open-the Meclodin® and Primperan® tablets (as references) were also determined using 900 ml of D.W at the same test environments. At preset time intervals, 5 ml samples were withdrawn and the filtrate was refluxed back using 5 ml of fresh dissolution medium. Samples were filtered through microfilter and analyzed spectrophotometrically at the λmax of MTB [8].

Factors Affecting Formulation:

Effect of Super Disintegrants Types and Concentrations on the Disintegration Time in the Mouth of the Prepared Control Orodispersible Tablets:

Formulas 1-12 (table 1) were utilized to study the effect of super disintegrant type (SSG, CCS and CP) and concentration (4, 8.5, 18 and 40 mg/tablet) on the disintegration time in the mouth of the prepared control orodispersible tablets. Formulas 14-16 were prepared to reveal the effect of super disintegrants combination at three different concentrations (1.65, 4.4 and 9.5 mg/tablet) on the disintegration time in the mouth of the prepared control tablets.

Effect of Diluents Types and Concentrations on the Disintegration Time in the Mouth of the Prepared Control Orodispersible Tablets:

Formulas 17-32 (table 1) were utilized to check the effect of diluent type (MCC101, MCC102, mannitol, lactose, L-HPC, DPD and glycine) alone and as combination of them at different concentrations on the disintegration time in the mouth of the prepared control orodispersible tablets.

Table (1): Composition of the Control Orodispersible Formulas

Material (mg) / Formula	SSG	CCS	CP	MCC 101	MCC 102	Mannitol	Lactose	L -HPC	DPD	Glycine	Mg St	Total Weight (mg)
1	4 *(2.5%)*			159							1	164
2	8.5 *(5%)*			159							1	168.5
Table (1): Continued.												

Material (mg) / Formula	SSG	CCS	CP	MCC 101	MCC 102	Mannitol	Lactose	L -HPC	DPD	Glycine	Mg St	Total Weight (mg)
3	18 *(10%)*			159							1	178
4	40 *(20%)*			159							1	200
5		4 *(2.5%)*		159							1	164
6		8.5 *(5%)*		159							1	168.5
7		18 *(10%)*		159							1	178
8		40 *(20%)*		159							1	200
9			4 *(2.5%)*	159							1	164
10			8.5 *(5%)*	159							1	168.5
11			18 *(10%)*	159							1	178
12			40 *(20%)*	159							1	200
13			54 *(25%)*	159							1	214
14	1.65 *(1%)*	1.65 *(1%)*	1.65 *(1%)*	159							1	164.95
15	4.4 *(2.5%)*	4.4 *(2.5%)*	4.4 *(2.5%)*	159							1	173.2
16	9.5 *(5%)*	9.5 *(5%)*	9.5 *(5%)*	159							1	188.5
17			40		159						1	200
18			40			159					1	200

Table (1): Continued

Material (mg) / Formula	SSG	CCS	CP	MCC 101	MCC 102	Mannitol	Lactose	L -HPC	DPD	Glycine	Mg St	Total Weight (mg)
19			40				159				1	200
19			40				159				1	200
20			40	80	79						1	200
21			40	80		69	10				1	200
22			40	80		79					1	200
23			40	59		100					1	200
24			40	40		120					1	201
25			40	20		140					1	201
26			40	20		140		2			1	203
27			40	20		140		23			1	224
28			40	20		140		50			1	251
29			40	20		140			11		1	212
30			40	20		140			23		1	224
31			40	20		140			50		1	251
32			40	20		140			23	55	1	279

Effect of pH on Conventional Disintegration Time of the Prepared MTB Orodispersible Tablet:

The effect of pH on conventional disintegration time of the prepared MTB orodispersible tablet was done using Erweka disintegration apparatus with 0.1N HCl solution (pH 1.2) and phosphate buffer (pH 6.5) as the disintegration media.

Results and Discussion

Effect of Super Disintegrants Types and Concentrations on the Disintegration Time in the Mouth of the Prepared Control Orodispersible Tablets:

Table (2) shows that the best super disintegrant type was CP with a concentration of 40 mg/tablet. This fact is represented in formula 12, which showed the fastest disintegration time in the mouth (27 sec). Furthermore, to ensure that the super disintegrant critical concentration of CP is 40 mg, another formula (formula 13) was made using 54 mg of CP. It was found that formula 13 disintegrated more slowly than formula 12 and showed mouth disintegration of 29.5 sec. On the other hand, the combination used in formulas 14-16 were not effective in lowering the disintegration time of the orodispersible tablets in the mouth since their disintegration time was relatively high and ranged from 40-60 sec. This may be caused by a competition between these super disintegrants on the little amount of water that found in the mouth knowing that 0.35-1.0 ml/min is the total volume of saliva available under normal conditions [10].

Table (2): The Effect of Super Disintegrants Types and Concentrations on the Disintegration Time in the Mouth of the Prepared Control Orodispersible Tablets *

No	Composition (mg)					Disintegration Time in the mouth (sec)
	SSG	CCS	CP	MCC101	Mg St	
1	4			159	1	44 ± 14
2	8.5			159	1	53 ± 18
3	18			159	1	66 ± 19
4	40			159	1	110 ± 27.3
5		4		159	1	45 ± 13
6		8.5		159	1	45 ± 14
7		18		159	1	42 ± 16
8		40		159	1	79 ± 24
9			4	159	1	58 ± 15.3
10			8.5	159	1	50 ± 20.7
11			18	159	1	39 ± 14.1
12			40	159	1	27 ± 5.1
13			54	159	1	29.5 ± 6.3
14	1.65	1.65	1.65	159	1	43 ± 7.8
15	4.4	4.4	4.4	159	1	40 ± 15
16	9.5	9.5	9.5	159	1	59 ± 18

*Results are expressed as mean ± S.D. (n=3)

Effect of Diluents Types and Concentrations on the Disintegration Time in the Mouth of the Prepared Control Orodispersible Tablets:

The super disintegrant concentration of CP (40 mg/tablet) was kept constant through out this part of the study to find out the best diluent that may be used for the preparation of control orodispersible tablet as shown in (table 3).MCC acts as auxiliary tablet disintegrant because of its water absorbing capacity [11]. The latter advantage gives the reason for the necessity for the inclusion of MCC in the formulation of orodispersible tablets. Practically, no difference was found between MCC 101 (formula 12) and MCC102 (formula 17) and they showed a mouth disintegration of 27 and 28 sec, respectively. Besides, combining two grades of MCC (MCC 101 and MCC 102) as in formula 20 gave slower disintegration time in the mouth (37 sec) than each grade alone.Table (3) also shows that mannitol was better than lactose in preparing

tablet with shorter disintegration time in the mouth. The mouth disintegration time for mannitol containing formula (formula 18) and lactose containing formula (formula 19) were 28 and 37 sec, respectively. This difference in disintegration time may be due to the fact that mannitol has slower dissolution kinetics, that is to say; lactose tends usually to dissolve rather than disintegrate, forming a viscous layer on the surface of the tablet which slows down the penetration of water (or saliva) into the tablet core [(12)]. On the other hand, formula 21 showed slower disintegration time in the mouth (39 sec) than both formulas 18 and 19 indicating that such combination is not effective in lowering the disintegration time in the mouth.Formulas 22-25 were utilized to study the effect of combining MCC 101 and mannitol at different concentrations on disintegration time in the mouth. Although formulas 22, 24 and 25 showed the same disintegration time in the mouth (26 sec), formula 25 was selected to complete the study because it contains the largest amount of mannitol which in turn, imparts sweet and cool taste on the prepared orodispersible tablet.Formulas 26-28 were utilized to study the effect of L-HPC on disintegration time in the mouth of the prepared control orodispersible tablet. It was found that L-HPC prolonged the disintegration time in the mouth at all concentrations, therefore, it can not be used in the preparation of orodispersible tablets.Formulas 29-31 were formulated using three different concentrations of DPD. Formula 30 was disintegrated faster than formulas 29 and 31 indicating that the best concentration of DPD is that used in the preparation of formula 30 (which is 23 mg/tablet and selected later to compete the study).Glycine is used in orodispersible tablet because of its effect as disintegration accelerator. Thus, the addition of glycine decreased the mouth disintegration of the tablet from 25 sec (formula 30) to 23 sec (formula 32). In addition, glycine has good flow and sweet taste, therefore, it was used in the past to hide the bitter taste of saccharine [(13)].Table (3) shows that the best control orodispersible formula (in terms of showing the fastest disintegration time in the mouth) was formula 32 since its disintegration time in the mouth was 23 sec.

Table (3): Effect of Diluents Types and Concentrations on the Disintegration Time in the Mouth of the Prepared Control Orodispersible Tablets (with out MTB) *

Formula	CP	MCC101	MCC102	Mannitol	Lactose	L-HPC	DPD	Glycine	Mg St	Disintegration time in the mouth (sec)
17	40		159						1	28 ± 4.9
18	40			159					1	28 ± 8.5
19	40				159				1	37 ± 12
20	40	80	79						1	37 ± 12.9
21	40	80		69	10				1	39 ± 7.4
22	40	80		79					1	26 ± 4.9
23	40	59		100					1	27 ± 6.4
24	40	40		120					1	26 ± 7.8
25	40	20		140					1	26 ± 7.0
26	40	20		140		2			1	34 ± 10.0
27	40	20		140		23			1	41 ± 9.0
28	40	20		140		50			1	43 ± 8.0
29	40	20		140			11		1	38 ± 7.8
30	40	20		140			23		1	25 ± 9.1
31	40	20		140			50		1	44 ± 11
32	40	20		140			23	55	1	23 ± 5.3

*Results are expressed as mean ± S.D. (n=3)

Formulation of MTB Orodispersible Tablet:

Formula 32 was the best control orodispersible formula, to which MTB, saccharine sodium and flavor were added. Table (4) shows the final MTB orodispersible formula (formula 33) which was subjected to further evaluation tests.

Table (4): Composition of the Selected (Final) MTB Orodispersible Tablet (formula 33).

Material	Amount (mg/tablet)
MTB	10
CP	40
MCC 101	20
Mannitol	140
DPD	23
Glycine	55
Saccharine sodium	2
Flavor	1
Mg St	1
Total weight	292

Physical Parameters Measurement of the Prepared Orodispersible Tablets:

Content Uniformity:

The content uniformity of the prepared MTB orodispersible tablet (formula 33) was complied with BP criteria. No tablet from ten tablets lies out of the range of 85-115% of the label claim. These results indicated that the dosage form had uniform distribution and proper dose of the active ingredient[8].

Hardness:

The hardness of all the prepared orodispersible tablets (with and without MTB) was kept constant at 4 kg by controlling the compression force between approximately 4-7 tone/cm^2.

Friability:

The friability of MTB orodispersible tablet (formula 33) was 0.5% which is acceptable according to BP criteria [8].

Disintegration tests:

Conventional Disintegration Time of the Prepared MTB Orodispersible Tablet (in vitro test):

The conventional disintegration test of the prepared MTB orodispersible tablet (formula 33) as well as Meclodin® and Primperan® tablets (as references) was determined using D.W as disintegration medium. The disintegration time of the prepared MTB orodispersible tablet was only 6 sec. In contrast, the disintegration time of Meclodin® tablet was 220 sec while that of Primperan® tablet was 120 sec as shown in figure (1). The disintegration time of the prepared MTB orodispersible tablet was also shorter than that obtained by other researchers[14].

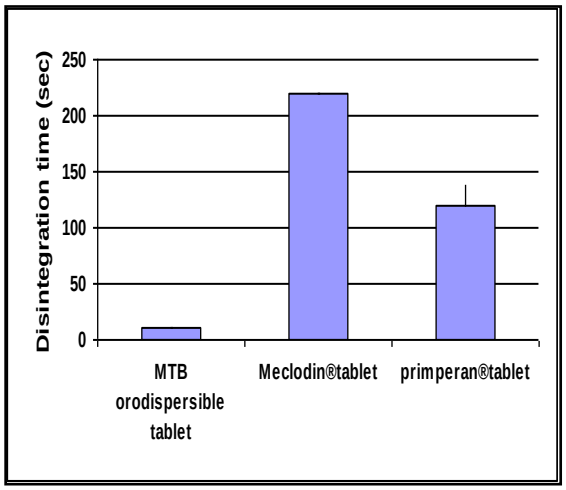

Figure (1): The disintegration time (conventional) of the prepared MTB orodispersible (formula 33), Meclodin® and Primperan® tablets using D.W at 37±0.5 ^{0}C (results are expressed as mean ± SEM, n=3).

Disintegration Time of the Prepared MTB Orodispersible Tablet in the Mouth (in vivo test):

The disintegration time of the prepared MTB orodispersible tablet (formula 33) in the mouth was found to be 23 sec (S.D=7.5, n=15). This result is quite satisfactory regarding BP criteria, which states that the disintegration time of orodispersible tablets in the mouth should not exceed 180 sec [8]. Not only acceptable in terms of BP, formula 33 had faster mouth disintegration than orodispersible tablets prepared by other researchers [15] and also faster than some of the commercialized products. For example, the disintegration time in the mouth of Benadryl® (USA), DuraSolv® (USA), Nimesulide® (Switzerland) and Nippon® (Japan) orodispersible tablets are 40, 45, 30 and 30 sec, respectively [16,17] as shown in figure (2).

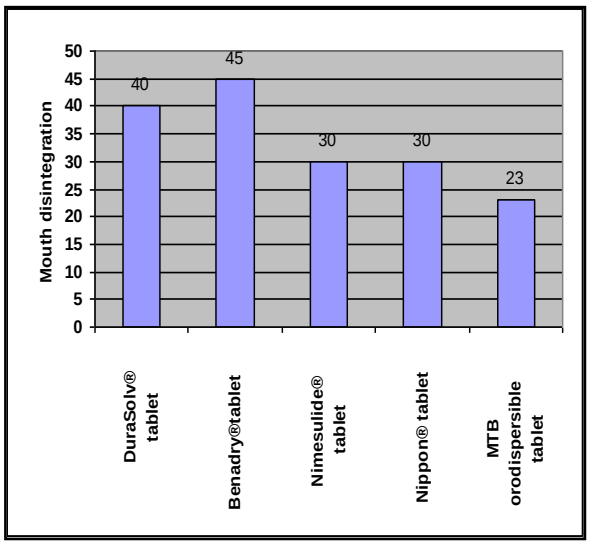

Figure (2): The disintegration time in the mouth of Benadryl®, DuraSolv®, Nimesulide®, Nippon® and the prepared MTB orodispersible tablets (formula 33).

Dissolution Test:

The dissolution test for the prepared MTB orodispersible tablet (formula 33) as well as Meclodin® and Primperan® tablets (as references) was done using 900 ml of D.W as a dissolution medium at 37±0.5 ^{0}C with constant stirring speed of 50 r.p.m for 30 min. Table (5) shows the result of the dissolution test.

Figure (3) indicates that the prepared MTB orodispersible tablet showed faster release rate than both Meclodin® and Primperan® tablets. The release time (T 75%) of the prepared MTB orodispersible tablet was 10-fold and 5-fold faster than that of Meclodin® and Primperan® tablets, respectively.Statistically, there is highly significant difference (P<0.05) among samples at 1, 2, 5, 10 and 15 min time intervals between Meclodin® and the prepared MTB orodispersible tablets. In addition, significant difference was found between samples at 1 and 2 min time intervals between Primperan® and the prepared MTB orodispersible tablets. These facts are clarified in figure (4).To accurately confirm the preference of the prepared MTB orodispersible tablet (formula 33) release profile compared to that of Meclodin® and Primperan® tablets, mathematical expressions had been used. To compare the dissolution profiles of two formulations (test and reference), the difference factor (F_1) and similarity factor (F_2) are useful. The F_1 and F_2 functions can be calculated according to the following equations [18]:

$$F_1 = \left(\frac{\sum_{t=1}^{n} |Rt - Tt|}{\sum_{t=1}^{n} Rt} \right) \times 100$$

$$F_2 = 50 \log \left\{ \left[1 + \frac{1}{n} \sum_{t=1}^{n} (Rt - Tt)^2 \right]^{-0.5} \times 100 \right\}$$

indicate clearly the high significant difference among these three formulations.

Table (5): The Percent Release of MTB from the Prepared Orodispersible (formula 33), Meclodin® and Primperan® Tablets in D.W at 37±0.5 ^{0}C *

Time Interval (min)	% Release of MTB from Meclodin® Tablet	% Release of MTB from Primperan® Tablet	% Release of MTB from the prepared MTB Orodispersible Tablet
1	32 ± 2.1 †	42 ± 3.1 †	77 ± 2.3
2	43 ± 2.1 †	55 ± 2.8 †	81 ± 2.3
5	48 ± 0.8 †	84 ± 6.6	88 ± 4.2
10	78 ± 2.1 †	96 ± 3.0	97 ± 1.9
15	95 ± 1.1 †	99 ± 0.5	99 ± 0.2
20	99 ± 0.4	100± 0.0	98 ± 0.8
30	100 ±0.0	99 ± 0.0	99 ± 1.0

* Results are expressed as mean ± S.D (n=3).
† Significant difference (P<0.05)

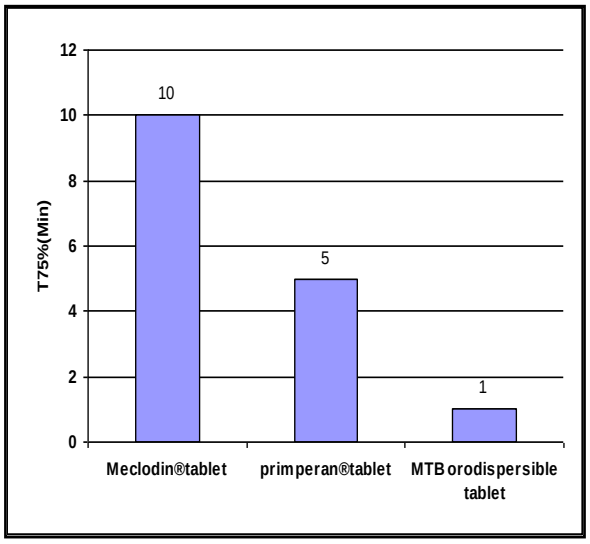

Figure (3): T 75% release of MTB from Meclodin®, Primperan® and MTB orodispersible tablets (formula 33) in D.W at 37±0.5 ^{0}C

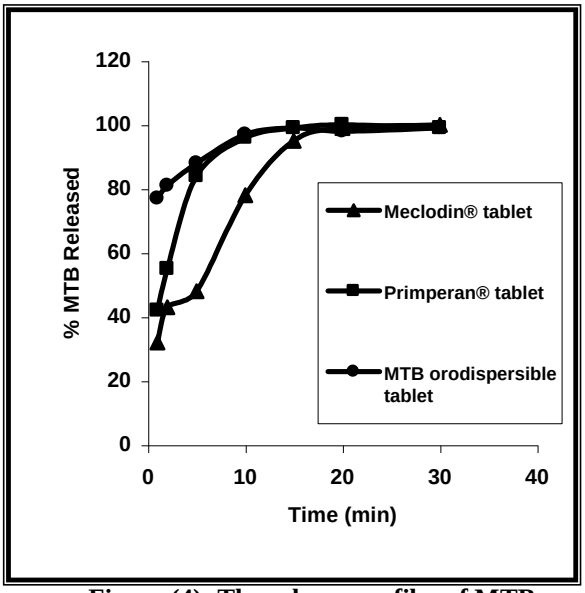

Figure (4): The release profiles of MTB from the prepared MTB orodispersible (formula 33), Meclodin® and Primperan® tablets in D.W at 37± 0.5 ^{0}C.

Effect of pH on Conventional Disintegration Time of the Prepared MTB Orodispersible Tablet:

Two ways are possible to administer orodispersible tablets. The first one is by putting the tablet in the mouth and waiting it to disintegrate without the aid of water. Alternatively, orodispersible tablet can be taken as a conventional tablet by using drinking water to push it down. Hence, two possible pH may face the tablet during its disintegration, which are either the neutral pH (6.5) in the mouth or the acidic pH (1.2) in the stomach [(19)]. Therefore, conventional disintegration test was made at these two pH so as to reveal the effect of pH, if any, on disintegration time of the prepared MTB orodispersible tablet and to ensure that this tablet disintegrates rapidly in all pHs. The effect of pH on disintegration time of the prepared MTB orodispersible tablet was undergone using Erweka disintegration apparatus with 0.1N HCl (pH 1.2) and phosphate buffer (pH 6.5) as the disintegration media. The disintegration time of the prepared MTB orodispersible tablet was 13 sec in pH 1.2 and 16 sec in pH 6.5 as shown in figure (4).It is obvious from these results that the disintegration time of the prepared MTB orodispersible tablet was not affected by changing the pH of the disintegration medium. This fact may be due that the super disintegrant (CP) used in the formulation of the prepared MTB orodispersible tablet does not affect by such change in the pH, therefore, tablet disintegration did not affect significantly by the shift of the pH [(19)].

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Determination of Cefalexin by Direct (UV- Vis) Spectrophotometer and Indirect(Flame Atomic Absorption) Technique

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Abstract

A new method for the determination of the drug cefalexin in some Pharmaceuticals using (UV-Vis) and indirect Flame Atomic Absorption Spectrophotometer (FAAS) , Fe III should forms a chelating complex with cefalexin (CEX –Fe III) at pH (1-8) and the best pH for the formation of (CEX –Fe III) chelating complex was (2) .The complex extracted with Methanol and Dimethy-Sulphoxide .The mole-ratio method has been used to determine the structure of chelate (CEX - Fe III) and found to be 2:1 LM (Ligand : Metal.) .

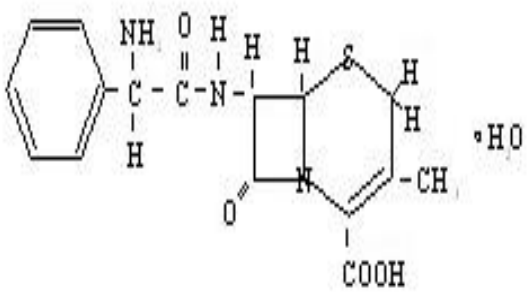
Keywords : Cefalexin , chelating complex.

الخلاصة

طريقة حديثة لتقدير السيفالكسين في بعض العقارات الصيدلانية و ذلك باستخدام تقنيتي الأشعة فوق البنفسجية-المرئية و الطريقة غير المباشرة للامتصاص الذري أللهبي .حيث وجد أن الحديديك يستطيع أن يكون معقد كيليتي مع السيفالكسين في مدى من قيم pH تتراوح ما بين (١- ٨) و أن افضل قيمة لـ pH تعطي اعلى ممتصية كانت عند (pH =٢) و المعقد الناتج استخلص باستخدام مزيج من الميثانول و ثنائي مثيل السلفوكساید و كانت النسبة المولية للتعقيد(CEX-FeIII) هي (٢:١) على التوالي.

Introduction

Cephalosporins are β –lactam antibiotics obtained originally From a cephalosporium mold.These antibiotics have the same mechanism of action as the Pencillins, but differ in antibacterial spectrum[1] . Cephalosporins discovered by Bortzu[2] in 1948. Cefalexin is the First generation of caphalosporins .The chemical names for cefalexin are :-7-(D – α -aminophenyl acetamido)des-acetoxy cephalosporic acid.[3] Or 7-(D-2-Amino-2-phenylacetamido)-3-methyl-3-cephem-4- carboxylicacid.[4] The chemical formula is C_{16} H_{17} N_3 O_4 S . H_2O . And the chemical structure for the drug :



Cefalexin monohydrate Molecular weight: 365.4 gm Melting point: 326.8 ^{0}C Cefalexin is a white to faint yellow powder slightly soluble in water, insoluble in ethanol , chloroform and ether . *Anacona et. al* [5] prepared complexes for β –lactam antibiotics with some metals, and they identify it by spectroscopic and physio-chemical methods ,also they studiedthe reaction of these antibiotics with some transitional elements like Mn II , Cu II ,Zn II, and confirmed the structures of products using I .R. and N.M.R. spectra. on the other hand, different solutions of cephalosporins and chloride salts of essential and trace elements prepared in a study using different temperatures (37˚Cand 60 ˚C) in order to accomplish the complex formation in molar ratio of the drug and metal salts 1:1[6]. Also *Abdel Gaber et.al* [7] studied the complexes of ions ZnII , CuII, Ca II with cephalosporins potentially they calculated the Molar – Ratio for (Liganed- Metal) at 25 ºC (1:1).

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Lozano and Borras [8] studied titrametically the complexes of Cefalexin with metals ZnII ,Cd II, they analysis the result by Electrical methods using (Least squares computer. program Super quad). They prepared complexes of cefalexin like Zn $(CEX)_2$, $3H_2O$ and Cd (CEX)(OH) H_2O , these complexes have been identified by I.R and N.M.R Spectra . In addition *Abo El-Maali et-al* [9] determined some cephalosporins antibiotic with Cd II or Zn II by using voltimeteric mothed which determined the presence of (liganed - Metal)1:1 like in complexes cefataxime , Cefuroxime and 2:1 like ceftazidime. Moreover, *Iqbal and Ahmad* [10] prepared complexes of cephalexin with copper II and ZnII these complexes have been characterized by microanalysis and by magnetic and spectroscopic analysis. The complexes, were found five-coordinate monohydrate, and ML_2 .On complexation with copper and zinc the antimicrobial activity of cephalexin improved Significantly. These results suggested that metallic elements should be seriously considered during drug design .Also, *Alekseev et al.*[11] studied the complex formation in solutions containing Nickel (Ni^{++}) cations,glycine anions (Gly -),and β-lactam antibiotics, they found mixed-ligand complexes is formed of [Ni Gly Ampicillin], [Ni Gly Amoxicillin] ,and [Ni Gly Cephalexin]. *Alekseev* [12], in another study, found that the complex formation of Neodymium III (Nd^{3+}) ions with Ampicillin, Amoxicillin and Cephalexin anions (L) in aqueous solution at 20˚C and ionic strength of 0.1 (KNO_3) by pH titration was in form of NdL and Nd(OH)L complexes using weak alkaline solution. A new study discussed the spectrophotometric determination of Cephalexin as intact cephalexin or its degradation product, cephalexin was determined in the range (1×10^{-5}-18×10^{-5} M) and the limits of detection were 0.3×10^{-5} M. These results were compared with reversed- phase HPLC determination, the UV-Vis spectrophotometric method was improved to be selective and reproducible [13].

Instruments , Materials and Method

A - Instruments

Shimadzu (AA- 670) Flame Atomic Absorption Spectrophotometer .

1. U.V–Visible Spectrophotometer(CARY 100)wave length 200 -1100 nm .
2. PH meter type 60 A .

B - Materials

All the chemical stock solution were prepared from analytical grade BDH,SDI ,India and Germany .

C- Method

Stock solution of Fe III

A solution of 1000 ppm of Fe^{+3} was prepared by dissolving 0.2896 gm of $Fecl_3$ in small amount of Water and complete the volume to 100 ml by using volumetric flask ,. Then 10 ml of the stock solution was diluted to 100 ml with distilled water to Prepare 100 PPMsolution .

stock solution of cefalexin (CEX)

A solution of 1000 ppm of CEX was prepared by dissolving 0.05 gm of (CEX) in water and complete the volume to 50 ml by using volumetric flask .Then a stock solution of 100 ppm was prepared as above .

Spectral study

Cefalexin spectrum

10 ml of stock solution (CEX) was transferred to a volumetric flask 5 ml and diluted with distilled water then the Absorbance was measured at 200-600 nm wave length using water as blank .

Ferric spectrum

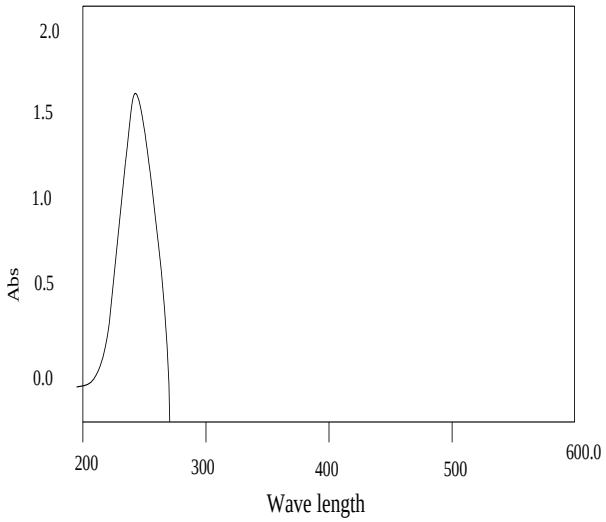
1ml of stock Ferric solution was transferred to a volumetric flask 5ml and diluted with distilled water, then the Absorbance was measured at 200-1100 nm using water as blank.

Formation complex with Fe III

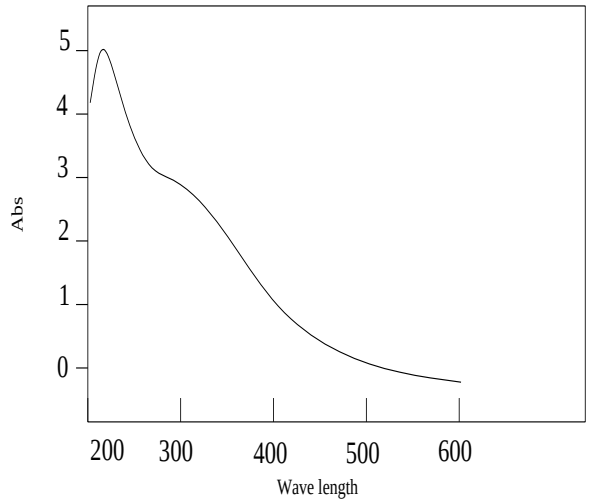
(2-5) ml from stock solution of (CEX) 1000 PPM was transferred to a volumetric flasks 5ml and 1ml of Fe III 1000 PPM was added .The chelating complexes can be extracted by using 2ml mixture of Methanol + Dimethyl sulphoxide (0.5 :1.5) .The formation of complex was stutied at room temperature The reaction was heated at different temperature with different pH .

Results and discussion

Drug spectra : measuring (CEX) spectra as in figure (1) we see one Absorption peak at λmax (264)nm using water as blank . Metal spectra : figure (2) for Fe III spectra show Absorption peak at λmax (208) nm using water as blank.Chelating complex spectra : CEX-FeIII.Figure(3) for chelating complex show two new peaks,the first at λmax (340) nm and the second at (358) nm which indicate the Formation of complex between drug and FeIII using organic solvent as blank. Table (1) show different compounds and the wave length at λmax for each drug and FeIII.



Fig(1) The UV absorption spectrum for cefalexin



Figure(2): The UV absorption spectrum for Fe III solution

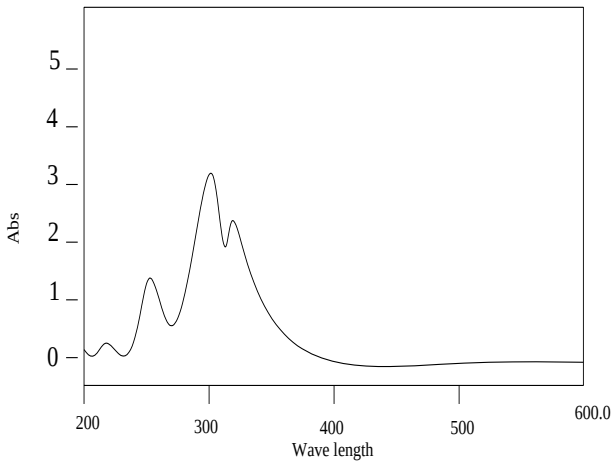


Figure (3) :The UV absorption spectrum for the complex CEX-FeIII

Table(1) Color and λ max for the drug FeIII and complex

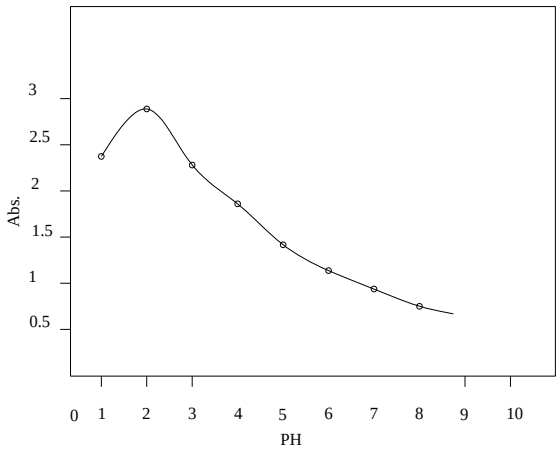
Compound	Max(nm)λ	Color
(CEX)	264	White
Fe III	208	Orange
Complex	341+358	Yellow

Optimum condition for complex formation

The experimental work showed that the reaction was not proceed at room – temperature so heating was necessary, media must be acidic, for these reasons we study the effect of pH, reaction time , extraction time, concentration of ion Fe III , temperature effect ,suitable solvent for extraction and number of extraction .

pH Effect:

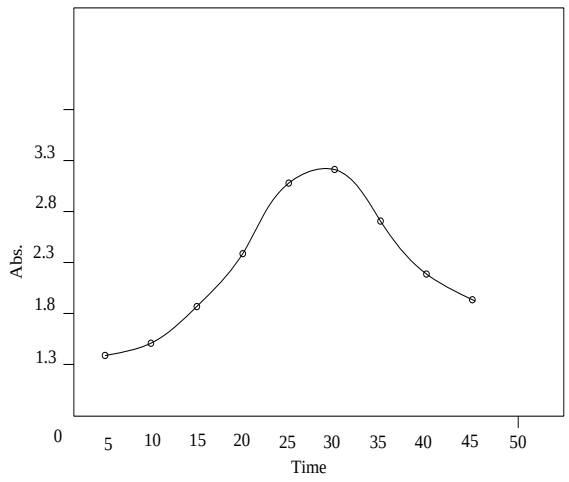
Figure (4) show the Absorbance of (CEX –Fe III) using different pH (1-8) the optimum pH for the complixation was (pH:2).



Figure(4) : Effect of pH on the absorbanc of CEX-FeIII by using UV spectrum

Reaction time

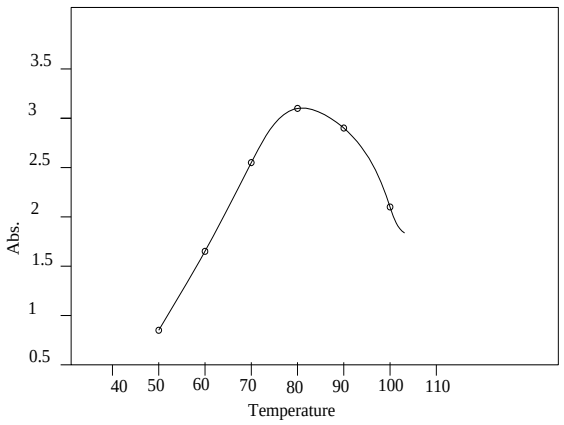
It was found that higher Absorbance of extracted complex was happened After 25 minutes, after that the Absorbance decrease due to dissociation part of complex With increase in heating time . Figure (5) , show that 25 minutes is the best time for reaction togive maximum Absorption for (CEX-Fe III) at 80ºC and pH 2.



Figure(5): Effect of reaction time on formation of complex CEX-FeIII at pH 2 by using UV Spectrum

Temperature effect:

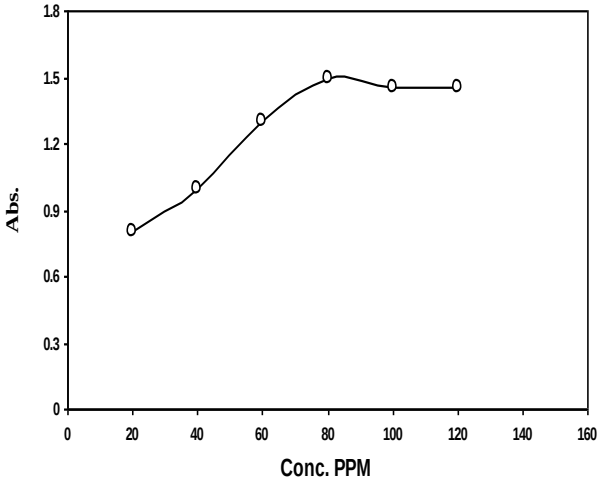
the reaction of drug with metal was proceed slowly it may exceed hours to increase the reaction velocity we proceed the reaction at different temperature (50-100 ºC), it was found that 80 ºC is the optimum temperature which gave the higher absorption as in figure (6) .



Figure(6) Complex absorption value at different temp. by using UV spectrum

Effect of Fe III concentration:

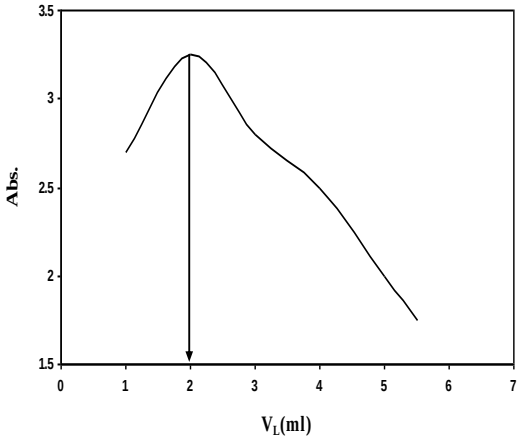
The best concentration for Fe III ion is 80 μg /ml, it gave the maximum Absorbance figure (7) show that effect of concentration PPM on absorbance .



Figure(7): The effect of Fe III Concentration on complex formation by using UV spectrum

Phase Ratio:

it was found 5ml of aqueous layer and 2ml of organic layer is enough to get higher Absorbance for complex as in figure (8), after that it was decrease as we increase the volume of organic layer.



Figure(8): Effect of phase ratio on absorbance of complex CEX-Fe III at pH =2 by using UV spectrum

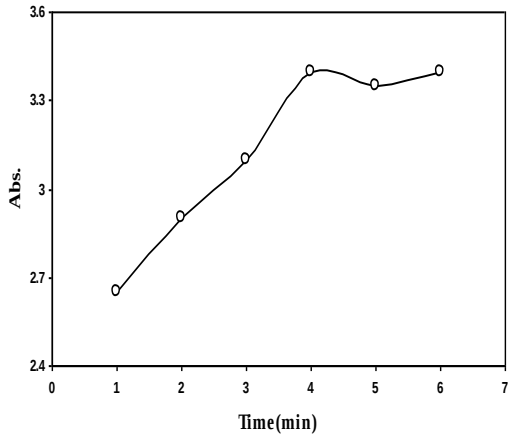
Suitable solvent :

Different organic solvent were used like Benzene, Chloroform ,Acetone Ethanol , Cyclohexanone , Carbon tetrachloride , Methanol , Dimethyl sulphoxide , and Diethyl ether for extraction of complex (CEX-FeIII) , it was found the mixture of Methanol and Dimethyl sulphoxide were the best solvents .

Extraction time :

The optimum time for shaking during the extraction of the complex was (4 min.) which give maximum Absorption . figure (9) showed

the maximum Absorption in the extraction was 4 minutes of shaking .



Figure(9) : Effect of shaking time on the extraction process by using UV spectrum

Number of extraction:

extraction process for once is enough to extract the complex ,because the second extraction for the remaining concentration give very small Absorbance less than 0.08 ,so one extraction is enough. This illustrate in table (2)

Table(2): Effect of number of extraction on absorbance of complex

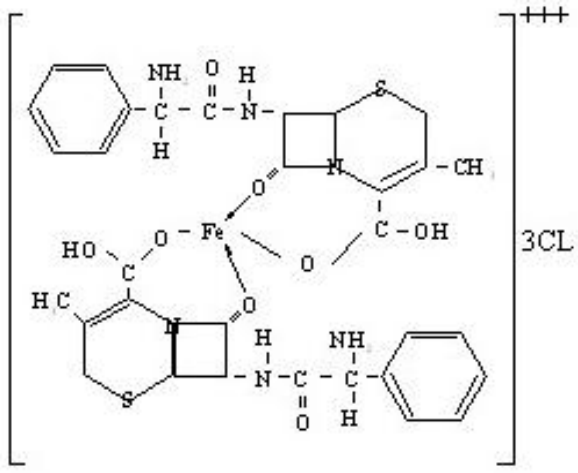
A0 Blank	0.005
A2 (Ex.no.2)	0.085
A1 (Ex.no.1)	2.84
PH	2
Fe III Mg/ml	60
CEX Mg/ml	800

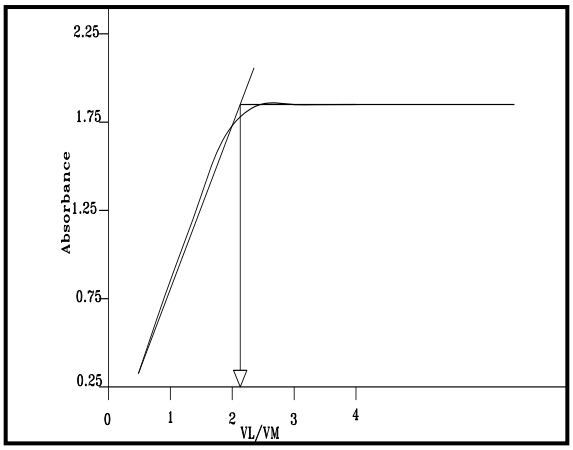
Mole Ratio of complex:

determination the mole ratio for complixation it means percent of moles of drug to the moles of metal , different volumes of CEX between (0.5-3.5)ml with constant volume of metal with all optimum conditions of complixation , it was found (1:2) (M:L) . Figure 10 show the relationship between absorbance . and V_L / V_M .The reaction of FeIII with drug occur in this equation :-

FeIII +2CEX ⟶ FeIII - 2CEX

The suggested chemical structure for the reaction of FeIII with CEX. :

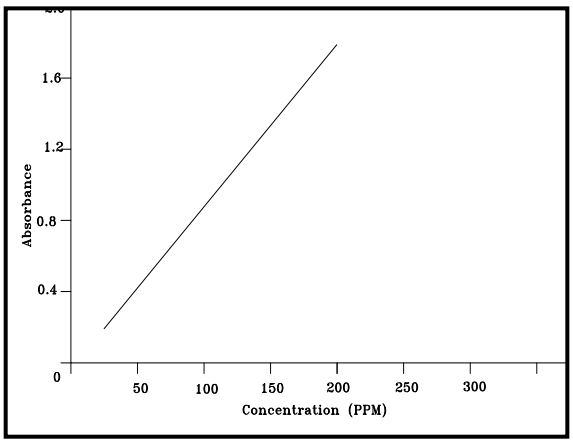




Fig(10) mole ratio of complex CEX-FeIII by using UV Spectrum

Calibration curve of the spectrum :

For determination of direct calibration curve for (CEX-FeIII),a different concentration for complex were used as in figure (11) and the relationship between Absorbance and concentration PPM was draw, it was found that the maximum concentration which obey Beer-Lumbert law 200μg / ml .



Fig(11) : Calibration curve for CEX-Fe III by using UV spectrum

Determination of the drug concentration in pharmaceutical preparation :

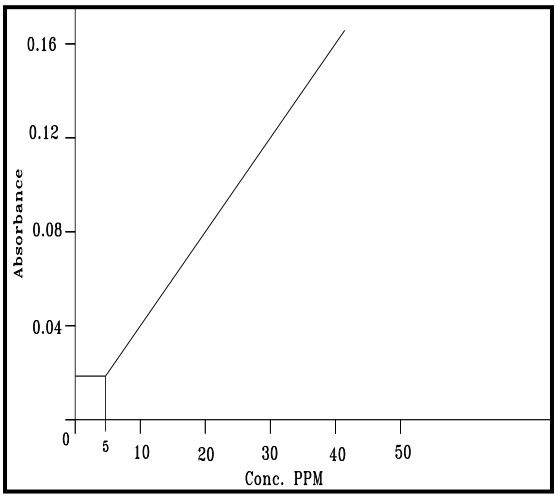
6 capsules were mixed with each other then one capsule was weighted , and the Absorbance was measured of its (CEX-FeIII) using optimum condition then from the calibration curve determine the concentration , we carried the procedure for CEX . (SDI ,Ajanta, Germany) .Table (3)show the result of work .From these result , we suggested that the CEX . concentration in Germany capsules is less than the real concentration which is 500 mg while Ajanta and SD Iis O.K.

Table(3):The concentration of Cefalexin capsule in 500mg cefalexin of different trade marks

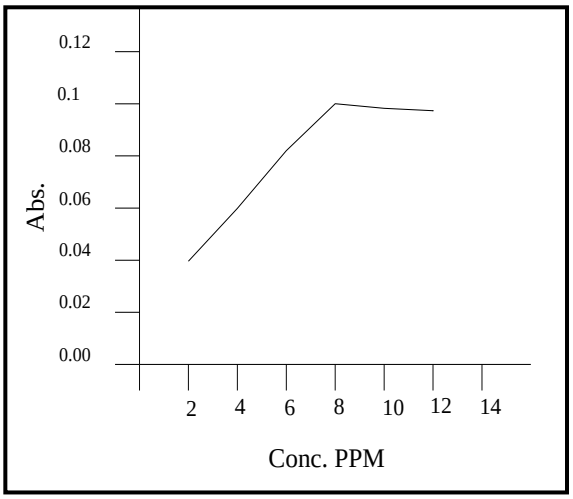
Germany	Ajanta	SDI	Capsules
1.29	1.3	1.4	Abs.
487	495	502	Conc.PPM
500	500	500	Standard

Determination of drug CEX – FeIII by using Flame Atomic Absorption Spectrophotometer:

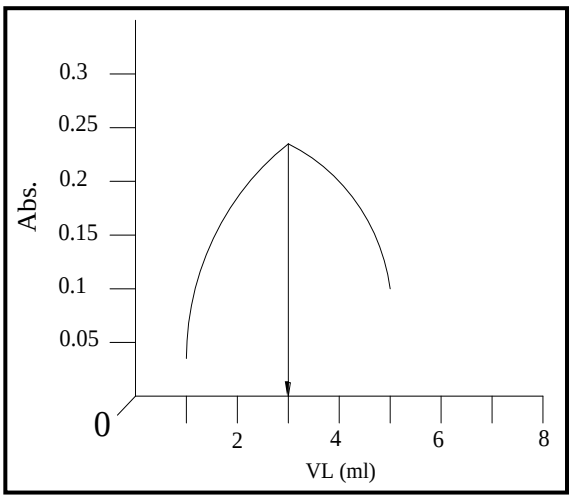
To be sure about the result obtained by U.V , we used another technical method, Flame Atomic Absorption Spectrophotometer (FAAS) , by indirect measurement the absorbance of Fe III in the complex to detect the cefalexin conc. as in figure (12) . The complex CEX-FeIII was prepared by using optimum condition of pH , temperature , proper solvent etc . (the same conditions mentioned previously in U.V spectro - photometer) except changing the conc.of ferric ion and phase ratio, it was found the best conc. of FeIII to give maximum absorbance 8 μg /ml , and 5ml of aqueous layer and 3ml of organic layer is enough to get higher absorbance for complex as in figure (13) and (14) .Also we measured the concentration of cefalexin in these pharmaceutical preparations using calibration curve of indirect (FAAS), we got the same result which obtained by U.V method



Fig(12) : Calibration curve for (CEX) by indirect FAAS by using UV spectrum



Fig(13): Effect of FeIII on complex by using FAAS



Fig(14): Effect of phase ratio on absorption of complex by using FAAS

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The Role of Chloroquine Phosphate on Acute Phase Reactant Proteins in Patients with Knee Osteoarthritis[#]

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Abstract

The acute phase response is a major pathophysiologic phenomenon that accompanies inflammation whether acute or chronic. Complement (C3 and C4) and C - reactive protein (CRP) are positive acute phase proteins (+ ve APPs). Their production takes place in hepatocyte and the blood concentration of these parameters are increased in osteoarthritis (OA). Chloroquine (CQ) is a diprotic weak base traditionally used to treat malaria. Recently the phosphate salt of CQ is used to decrease this type of (+ve APPs) . In this study, patients who suffered from knee osteoarthritis (KOA) are treated with oral dosage form of chloroquine phosphate (CQP) for one month, twice daily. Our results demonstrate that CQP improves the patient status by decreasing complement and C-reactive protein in blood.

Key words: Chloroquine , knee osteoarthritis , acute phase proteins , complement , C-reactive protein.

الخلاصة

لقد تمت دراسة دور عقار فوسفات الكلوراكوين على مستوى البروتينات التفاعلية CRP, C4, C3 لدى المرضى الذين يعانون من الفصال العظمي للركبة.أجريت تجربة عشوائية على 50 شخصا من الاصحاء (30أنثى , 20 ذكر) و 74 مريضا (45 انثى و 29 ذكر) في مستشفى مدينة الطب العيادة الاستشارية من(شهر كانون الثانى الى ايلول 2008) تم تشخيصهم من قبل الطبيب الاختصاصي حسب طريقة الكليه الأمريكيه لمبحث الرثيه حيث تم تحريز الأعراض بواسطة مؤشر (كليكرين ولورنس) للفصال العظمي ، أخذت صور شعاعيه أماميه خلفيه لمفصل الركبه وحدد مستوى البروتين التفاعلي- C مع مستوى كل من ألمتممين C3 و C4. وصف لهم حبوب فوسفات الكلوراكوين بتركيز (250 ملغم) مرتين باليوم ولمدة شهر. سحبت عينة من دم الاصحاء والمرضى اولاً ثم عينة من دم المرضى الذين استعملوا هذا العقار بعد شهر لغرض قياس نسب البروتينات التفاعلية في المصل. لوحظ انخفاض مستويات هذه العوامل بصورة واضحة ومعنوية عند استعمال العلاج حيث زيادتها تلعب دورا مهما في تسريع تآكل الغضاريف وتكوين النتوءات العظمية.

Introduction:

OA is the most common disease in the world and a major cause of pain and disability which usually develops in distal inter phalangeal (DIP)joints of finger, the weight bearing joints of leg and the movable portion of spine[(1)]. It is associated with a breakdown of cartilage in any joint in the body[(2)]. Pathologically, OA was defined as a gradual loss of articular cartilage combined with thickening of subchondral bone/ bony out growth (osteophyte) at joint margins with mild to chronic non specific synovial inflammation[(3)]. CQ is 4 -aminoquinoline approved for treatment and prophylaxis of malaria. Recently CQP is used by some authors in the OA as disease modifying anti –rheumatic drug (DMARD) [(4)], claimed to cause lowering of blood level of proinflammatory mediators [(5)] . Our study shows the effect of CQP on the serum concentration of CRP, C3 and C4 in patients with KOA.C-reactive protein is a laboratory marker that is important in the assessment of inflammation, serve as a predictor and indicator of response to therapy and overall outcome in various disorders [(6)]. The major function of CRP is to bind phosphocholine, this is by permitting recognition of foreign pathogens and phospholipid constituents of damaged cells [(7)], so the activation of complement system and / or binding to phagocytic cells will take place , and to initiate elimination of targeted cells by interaction with both humoral and cellular affecter system of inflammation , as aresult CRP is a component of innate immune response [(8)],and useful in early detection of low grade inflammation[(9)].

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C3 and C4 serve proinflammatory roles, including chemotaxis, plasma protein exudation at the site of inflammation and opsonization of infectious agents and damaged cells[10].C3 is beta - 2 protein 180 KDa , cleave by C3 convertase into C3a and C3b. C3b reacts with factor B to produce more C3 convertase and activate C5, so the serum level of C3 is increased in acute inflammation[11]. C4 is beta -1 protein, 210 KDa cleave by C1s to produce C4a and C4b, C4b interact with C2b to activate classical pathway C3 convertase. [12]

Patients and method

Fifty healthy people (30 female and 20 male) as control and seventy - four patients (45 female and 29 male) are selected randomly from the Out Patient Clinic in Baghdad Teaching Hospital / Medical City / Baghdad from January to September 2008 with inflammatory KOA diagnosed according to American College of Rheumatology[13]. All patients were assessed by Kellegren and Lawrence grading criteria for radiographic severity of knee osteoarthritis with different signs and symptoms such as joint pain, stiffness, bony enlargement, bony tenderness and crepitus[14]. Their ages are ranged from (45 to 78) years with mean value ± standard error of mean (55.07 ± 6.18).Chloroquine phosphate tablet (Medoquine 250mg/ Medochem Company is equivalent to 150mg CQ base), was prescribed by Rheumatologist as twice daily after meal for one month,because CQP is 4-aminoquinoline derivative(CQ+phosphate) ,it absorbed completely and rapidely from GIT ,its mean absorption half –life is four hours with a lag time slightly more than 30 minutes [15,16],and its duration of action is extended from sixteen to forty five days [17].The serum analysis of all patients and control was done in the General Health Laboratory Center / Baghdad, before using this drug and one month later in order to estimate the level of C3, C4 and CRP.C3 and C4 are determined by Radial Immune Diffusion[11] while CRP is assessed turbidit-metrically by antigen antibody reaction technique [17] . Results were analysed by statistical package for social science (SPSS).

Results

The presented data in this study showed the comparisons between level of C3,C4 in microgram per milliliters (µg/ml) and CRP in milligram per liter(mg/ l) in healthy and patients before the treatment as well as after one month of using chloroquine phosphate are depicted in table (1),figures(1,2,3).There were a significant decrease in C3,C4 and CRP in male,female and total patients compared to their level at baseline and control.

Table (1): The serum level of C3,C4 and CRP before using CQP at baseline and after one month of the treatment.

		Control	Baseline	P value Control, Baseline	After one month	P value Pre-,post-treatment
*C3 (*µg/ml)	T M F	950.1±21.1 877.3±17.3 987.4±20.2	*1794.4*±34.2 1788.2±48.8 1798.4±47.16	S p>0.01	1504.5±31.1 *1503.4*±40.9 1505.3±41.7	S P<0.05
C4 (µg/ml)	T M F	320.23±12.2 318.1±15.7 307.2±13.4	396.08±14.6 367.7±20.2 414.3±19.9	S p>0.01	325.03±12.7 288.03±17.1 323.09±17.9	S P<0.05
CRP (MG/l)	T M F	1.08±0.1 1.1±0.12 2.09±0.17	4.3±0.3 3.8±0.6 4.63±0.4	S p>0.01	2.02±0.2 1.8±0.37 2.1±0.23	S P<0.05

SEM: Standard error of mean.
Significant P value : (P<0.05),(P>0.01).

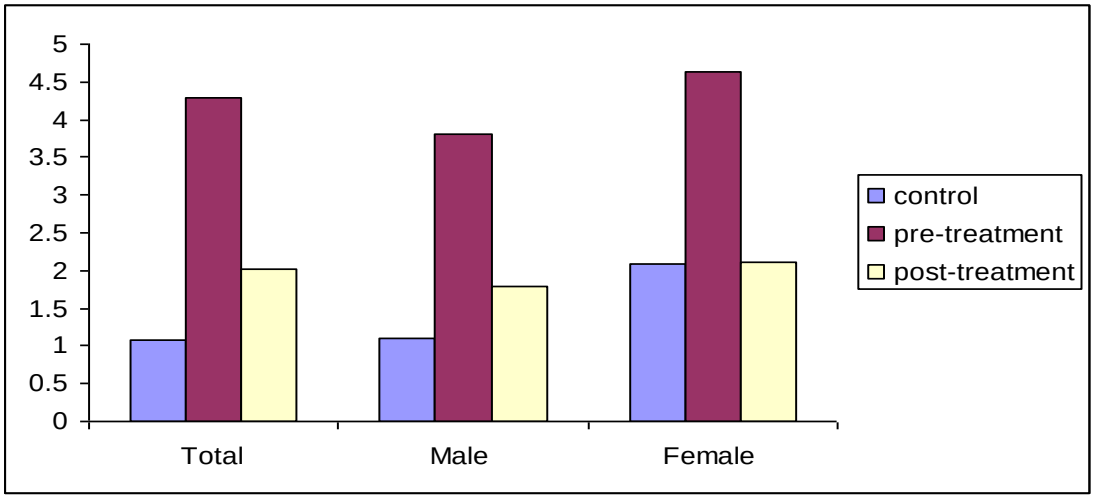

Figure (1): The level of serum C-reactive protein at baseline (pre-treatment), after one month of using chloroquine phosphate (post-treatment) and control.

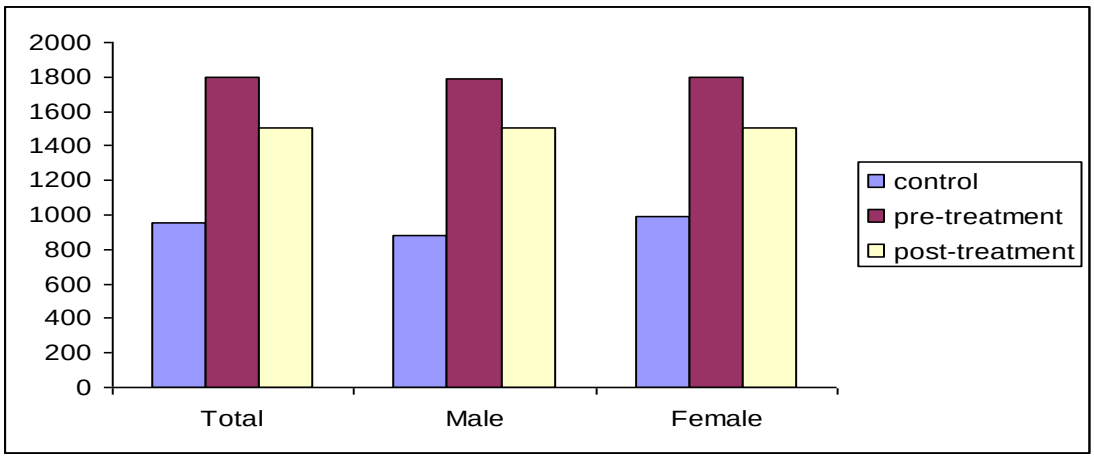

Figure (2): The level of serum complement three at baseline and one month later of using chloroquine phosphate.

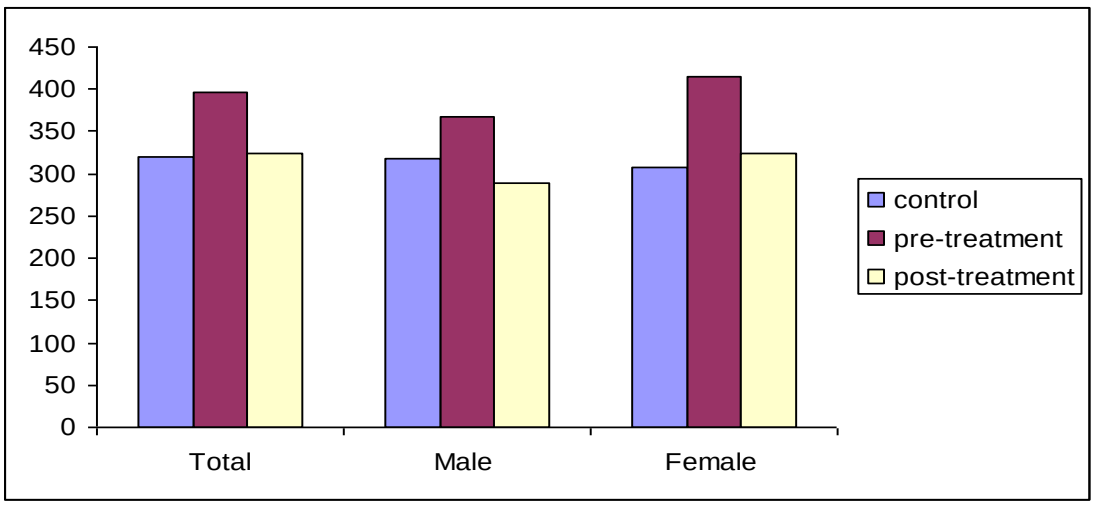

Figure (3): The level of serum complement four at baseline and one month later of using chloroquine phosphate.

Discussion

C3, C4 and CRP are components of +ve APPs , their production are increased by hepatocyte [18]. The elevation of these proteins are detected in patient with OA which is due to releasing of inflammatory molecules (cytokines)[19]. Jawad et al in 2004[4] demonstrated that the using of CQP as DMARD for three months in patients with KOA, lead to decrease the serum CRP level. In our study the presented data shows a significant decrease in this parameter after one month of using CQP ($p<0.05$), table (1), figure (1), so our result is in agreement with all explanation and findings. Chloroquine phosphate decreases serum level of CRP, C3 and C4 depending on it's ability to enter lysosomes and all acidic compartments of the cells (lysosomotropic effect)[20]. It interferes with intracellular processing, receptor recycling[21] and the secretion of proteins which lead to decrease the production of cytokines and other inflammatory mediators decreases lymphocyte proliferation as an immune effect[22]. Non - lysosomotropic effect of CQP includes the inhibition of phospholipase, antagonization of prostaglandin stabilization of lysosomal membrane in synoviocytes [23,24,25]. In 2006 , Numman et al used silymarin to treat patients with KOA instead of CQP[26]. Silymarin is a plant (mixture of flavolignans),isolated from the ripe seeds of Silybum marianum (Milk Thistle), it proved to have effective inhibitory effects on cyclooxygenase and 5-lipooxygenase in vitro and experimental animals[27]. Their results showed that this drug when prescribed for two months in KOA, it decreases the serum level of C3 and C4 significantly. Our study shows a significant decrease in C3 and C4 at the end of trial ($p<0.05$) table (1), figure (2) and (3). All findings, trials in addition to the mode of action of CQP are supported the results.

Conclusion:

CRP, C3 and C4 are decreased after using CQP for one month in patients with KOA.

Recommendation

Further study is needed to asses other parameters in serum and synovial fluid.

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The Effect of Atenolol on CK-MB Levels in Hypertensive Patients[#]

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Abstract

Atenolol is one of beta-adrenergic receptor blocking agent. It is widely used for the treatment of hypertension as a selective antihypertensive drug. But long term usage of atenolol may cause one of the cardiovascular diseases like myocardial infarction. To prove the relationship between atenolol and cardiovascular disease, measurement of creatinekinase-MB as a diagnostic indicator in early and long term usage of this drug by hypertensive patients is recommended. A comparative study was conducted in Al-Yarmouk Teaching Hospital–Emergency Department- on 30 hypertensive patients using atenolol. They were divided into (2) groups A and B according to the duration of the drug usage. Group A- (15) patients with a mean age (56±6) years. They used atenolol for a period of (1-5) years. Group B- also (15) patients with mean age (60±6) years. They used atenolol for (6-20) years. Both groups were with nearly the same number of males and females. All subjects of the study groups were screened to exclude evidence of hyper or hypothyroidism, diabetes and chronic renal failure. Venous blood samples were taken in first 8 hours after onset symptoms of cardiac attack from each patients and the levels of creatine kinase-MB were estimated and compared between the (2) groups. There is a significant correlation between levels of serum creatine kinase-MB of group A and group B ($P<0.05$). Atenolol causes increased level of serum CK-MB and this increase was directly proportional to the duration of the drug usage. CK-MB is one of cardiac markers that released from heart muscle when it is damaged as a result of myocardial infraction. So, atenolol has a significant correlation with development of myocardial diseases.

Key words: hypertension , Atenolol and side effects , creatine kinase-MB , Atenolol antihypertensive.

الخلاصة:

التينورمين هو واحد من مجموعة الأدوية التي تعمل على غلق مستقبلات بيتا، وهو يستخدم بشكل واسع في معالجة ارتفاع ضغط الدم ولهذا فإن هذا العقار قد يصنف كدواء اختياري لتقليل ارتفاع ضغط الدم ولكن استخدام هذا العقار على مدى طويل قد يسبب حدوث واحد من امراض الجهاز القلبي الوعائي. ولإثبات العلاقة بين عقار التينورمين وأمراض الجهاز القلبي الوعائي بواسطة قياس أنزيم الكرياتين كاينيس م ب ككاشف تحليلي على المدى القصير والبعيد لاستخدام هذا العقار لدى مرضى ارتفاع ضغط الدم، ولهذا أجريت دراسة مقارنة حالات في مستشفى اليرموك التعليمي- قسم الطوارئ- على (٣٠) مريضاً يعانون من ارتفاع ضغط الدم ويستعملون تينورمين كعلاج وتم تقسيم هؤلاء المرضى إلى مجموعتين (أ وب) وفقاً إلى طول فترة استعمالهم لعقار التينورمين. المجموعة (أ) وتتألف من (١٥) مريضاً، معدل أعمارهم (٥٦±٦) سنة واستعملوا التينورمين لفترة زمنية تراوحت بين (١-٥) سنة والمجموعة (ب) وتتألف أيضاً من (١٥) مريضاً، معدل أعمارهم (٦٠±٦) سنة استعملوا التينورمين لمدة تراوحت بين (٦-٢٠) سنة. كلا المجموعتين تقريباً تضم نفس العدد من كلا الجنسين، وكل المرضى المشمولين بهذه الدراسة غير مصابين بأمراض الغدة الدرقية ولا بداء السكري أو عجز الكلية المزمن. تم أخذ عينات من دم كل مريض خلال الساعات الثمانية الأولى بعد ظهور أعراض الأزمة القلبية لقياس مستوى أنزيم الكرياتين كاينيس م ب وتمت مقارنة النتائج بين المجموعتين. كان معدل الأنزيم كرياتين كاينيس م ب في مصول دم المجموعة (ب) مقارنةً بالمجموعة (أ) عالية وبمعنوية واضحة $P< ٠,٠٥$. ومن خلال النتائج التي تم الحصول عليها تبين أن عقار التينورمين يؤدي إلى ارتفاع وزيادة مستوى الأنزيم كرياتين كاينيس م ب وهذه الزيادة تتناسب طردياً مع طول الفترة الزمنية لاستعمال التينورمين وبما أن هذا الأنزيم مؤشر حيوي خاص لأمراض الجهاز القلبي الوعائي، لذا فإن عقار التينورمين له علاقة معنوية بحدوث أمراض الجهاز القلبي الوعائي وخصوصاً احتشاء العضلة القلبية.

Introduction:

Atenolol is one of beta blockers acts by blocking beta receptors that are found in various parts of the body, and prevents the action of nor-adrenaline and adrenaline[(1)]. Atenolol is rapidly absorbed from the gut. Blood level reached a peak concentration in (2-3) hours[(2)]. Metabolism of atenolol is minimal and almost the total absorbed drug (85-100)% is cleared via excretion in the urine in an unaltered manner[(3)]. Although atenolol is the drug of choice in different cardiovascular diseases as angina pectoris, hypertension, arrhythmias and in prevention of heart attack[(4)]. The prolong use of this drug as antihypertensive may show different side effect which may develop to symptoms of cardiovascular disease. Creatine Kinase-MB is one of the isoenzymes of creatine kinase which is mostly found in the heart. I measured creatine kinase-MB as an important biological marker, when it appears in abnormal level>10u/L in serum.

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This means that there is a myocardial injury. CK-MB shows increases above normal in a person's blood test about four to six hours after the start of a heart attack. It reaches its peak level in about 18 hours and returns to normal in 24 to 36 hours[5]. CK-MB is both a sensitive and specific marker for mycocardial infarction, most commonly used to confirm the existence of heart muscle damage.

Materials and Method:

This comparative study was done in the Emergency Department in Al-Yarmouk Teaching Hospital on (30) hypertensive patients (48-68) years who received atenolol tablet 100mg as antihypertensive drug for a duration of (1-20) years. The patients were divided into (2) groups according to the duration of drug use:

Group A: consists of (15) patients with a mean age (56±6), they used atenolol for a period of (1-5) years.

Group B: consists of also (15) patients with a mean age (60±6), they used atenolol for a period of (6-20) years.

Venous blood samples were obtained from each patient of both groups for measuring the level of CK-MB. The method used for measuring CK-MB is Immunoinhibition Assay (RANDOX) in which an antibody is incorporated in the CK reagent. This antibody will bind to and inhibit the activity of the M subunit of CK-MB. This means that only the activity of the B subunit in serum is measured [6,7]. The sample is serum, heparinized or EDTA plasma. Haemolysis interferes with the assay. Reagents are a mixture of CK-MB Buffer/Glucose (Imidazole Buffer, Glucose, Mg-Acetate and EDTA) with Enzymes/Coenzymes/Substrate/Antibody (ADP, AMP, Diadenosine pentaphosphate, NADP, HK, G-6-PDH, N-Acetylcysteine, Creatine Phosphate and Antibody to CK-M).
A patient sample is added to the reagent mixture read the absorbance directly at 340nm (A_1), the second reading is after five minutes exactly (A_2). $\Delta A = A_2 - A_1$
ΔA multiplied by 1651 (kit factor) gives the concentration of CK-MB in u/L. This procedure is done at room temperature 25°C.

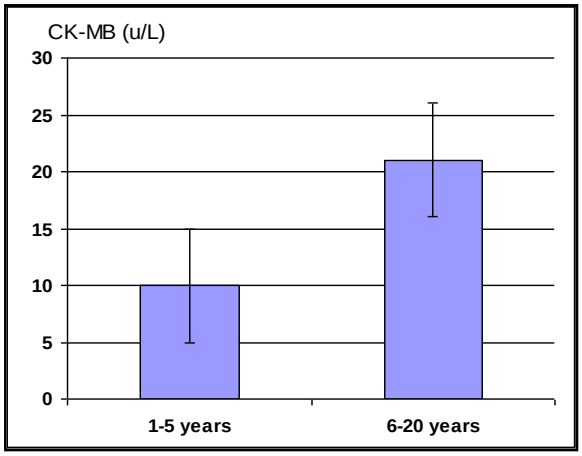
Results:

After collection and categorization of data from the (30) patients included in the study, statistical analysis was done [table 1 and Fig.1] which revealed the following:

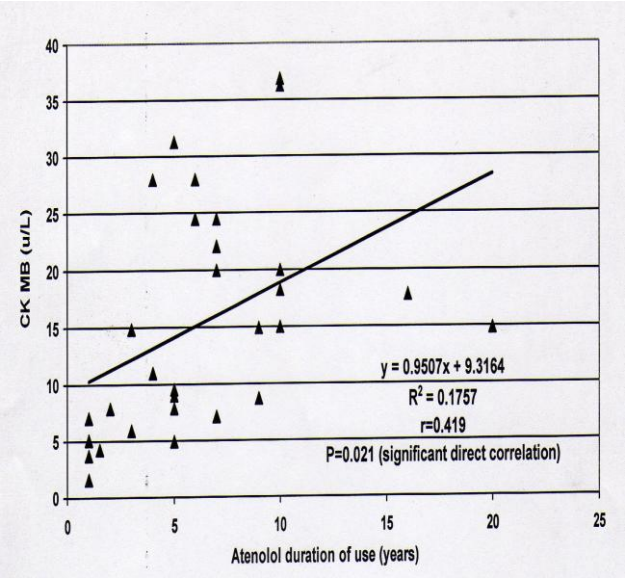
1. The correlation between atenolol duration 1-5 years and CK-MB (u/L) in patients included in the study ($y = 2.4336x+2.5759$, $R^2 = 0.236$, $r=0.486$, $P=0.066$ (Not significant)).
2. The correlation between atenolol duration 6-20 years and CK-MB (u/L) in patients included in the study ($y= -0.3751x+24.188$, $R^2= 0.277$, $r= -0.166$, $P= 0.553$ (Not significant)).
3. The correlation between atenolol duration (years) and CK-MB (u/L) in total 30 patients included in the study ($y=0.9507x+9.3164$, $R^2=0.1757$, $r=0.419$, $P=0.021$ (significant direct correlation) as shown in Fig. (2).

Table(1) : The CK MB(u/L) concentration duration of use of atenolol in hypertensive included in the study

Atenolol duration of use (years)		1-5 years	6-20 years
CK MB (u/L)	Mean	10.12	20.59
	SD	8.58	8.60
	Minimum	1.6	7.1
	Maximum	31.3	36.9



Figure(1) : Correlation between time of atenolol usage and serum CK-MB



Figure(2) : The correlation between the duration of atenolol treatment (years) and CK-MB (u/L) in total 30 patients included in the study

Discussion:

Atenolol is widely used all over the world for the treatment of hypertension. It is an efficient antihypertensive but it has many side effects which sometimes they might be serious. Enzymology is a diagnostic indicator for cardiovascular disease in hypertensive patients with atenolol treatment[8]. CK-MB, the primary indicator used to diagnose a heart attack because it exists in the highest amount in the heart helps in converting creatine to creatinine, a reaction that is necessary for metabolism and energy production. So, the level of CK-MB determines the effectiveness of antihypertension drug which provides a diagnostic clinical evidence[8]. Rise in the level of this enzyme (CK-MB) has been reported in hypertension with myocardial infarction patients[9,10]. Enzymes always have been identified as a specific and sensitive markers of both clinical and subclinical myocardial injury[11]. Therefore biological marker like CK-MB to quantify myocardial injury has been widely used in clinical practice. In cardiac muscle they are tightly bound to the contractile apparatus and therefore plasma concentrations is extremely low. With acute myocardial injury, there is release of CK-MB into the serum, the extent of the elevation in serum depends on the severity of the myocardial injury. And the entry of this enzyme in circulation depends upon the rate of passive diffusion of the enzyme from infarct myocardium cells[12]. One of the most reliable and commonly tested cardiac enzyme is CK-MB which released specifically from injured heart muscle[13].Increased serum levels of CK-MB in hypertensive patients taking atenolol is directly proportional to the duration of the atenolol usage. Long exposure of cardiac muscle to atenolol leads to escape of CK-MB to circulation. The mechanism by which atenolol causes myocardial injury is not yet known and this may be due to cardiac muscle which becomes fatigue with prolonged exposure to atenolol causing it unable to contract efficiently and ending with failure[14].

Conclusion:

Atenolol should be used selectively and in acute urgent cases for different cardiac diseases. For hypertensive patients of long term usage checking should be followed continuously to make sure if any symptoms of cardiac injury appears and in such a case terminates using atenolol and other antihypertension drug should be described.

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Spectroscopic Study for Determination of Amoxicillin Using Cobalt(II) as Complexing Metal

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Abstract

This study includes analytical methods for the determination of the drug amoxicillin trihydrate (Amox.) in some pharmaceutical preparations using Cobalt ion (Co(II)) as complexing metal. The best conditions for complexation were: the reaction time was 20 minutes, pH=1.5 and the best temperature of reaction was 70 ˚C. Benzyl alcohol was the best solvent for extraction the complex.

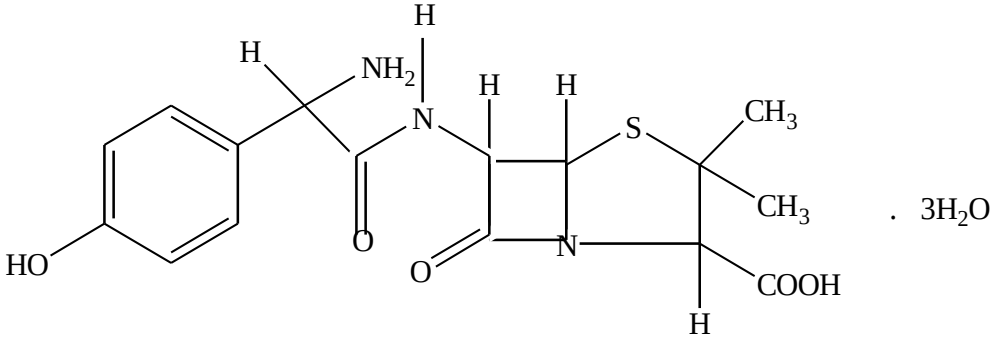
Keywords: Amoxicillin, Cobalt(II), Complex, Molar ratio.

الخلاصة

يتضمن البحث استحداث طرق تحليلية جديدة في تقدير المركب الدوائي اموكسسلين ثلاثي جزيئات الماء (Amoxicillin Trihydrate) وذلك بتكوين معقد للعقار مع أيون الكوبلت (Co II) حيث وجد أفضل pH لعملية التعقيد عند دالة حامضية (pH=١,٥) وان أفضل درجة حرارة لعملية التعقيد مابين العقار والايون الفلزي كانت بحدود ٧٠ درجة مئوية, وأفضل زمن للتفاعل فكان بحدود ٢٠ دقيقة,قد جربت عدة مذيبات حيث وجد إن الكحول البنزيلي هو أفضل مذيب لعملية الاستخلاص.

Introduction

Amoxicillin is one of the important derivatives of semisynthetic penicillin; it is active against Gram positive and to less extent Gram negative bacteria. Its nomenclature according to penicillins is 6-[D(-)-α-Amino-p-hydroxyphenyl acetamido] penicillanic acid or α- amino-p-hydroxy benzyl penicillin[(1)] ,while its systematic (IUPAC) name is 7-[2-Amino-2-(4-hydroxypHenyl)-acetyl]amino-3,3,dimethyl-6-oxo-2-thia-5- azabicyclo [3, 2, 0] heptane-4-carboxylic acid, the chemical structure of the drug is [(1)]



The formula structure of Amoxicillin as trihydrate (drug) is $C_{16}H_{19}N_3O_5S.3H_2O$, its molecular weight = 419.45 gm.mole^{-1}. It is off white or almost white crystalline powder, slightly soluble in water and alcohol such as methanol and ethanol [(2)]. It has UV max. (ethanol): 230,274 nm and in (0.1N HCl): 229,272 nm [(1)].Imran et al. prepared complexes of amoxicillin with Zn(II), Cu(II), Ni(II)and Ag(I), they identify these complexes by (C, H, N)elemental analysis and IR Spectra. These complexes have increased the biological activity of the drug [(3)]. Jian et al. determined amoxicillin in tablets, they used a quick and simple method which is (second differential derivative) at λ_{max}. 282 nm and the standard deviation was less than 1.2% and the standard recovery for the drug was 97-100.5 % [(4)].Denis et al. determined amoxicillin and clavulanic acid in blood plasma by HPLC supplied by UV detection, and they found the linearity was (0.62 – 20 µg.ml^{-}) while the detection limit for amoxicillin was 0.312 µg.ml^{-} [(5)].

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Ashry et al. detected phenolic antibiotic like amoxicillin by its reaction with benzocaine in the presence of triethylamine at $\lambda_{max.}$ = 455 nm the linearity was (2 – 16 µg.ml^{-1}) while the detection limit was 0.0034 µg.ml^{-1} [6].Co(II) forms blue-colored complex in the organic phase with Cyanex 923, a sensitive analytical reagent, the $\lambda_{max.}$ of the complex was 635 nm and the concentration that obeyed Beer's law is (58.9-589.0 µg. ml^{-})[7].Zayed et al. in a new study, prepared different complexes of amoxicillin with Zn (II), Ni (II), Co (II) and Cu (II), these complexes were studied using elemental analysis, IR and mass spectra. The molar ratio of complexes were found to be Metal:Drug =1:1,1:2, and the stability constant K_f of these chelates was(10^7-10^{14}) [8].In a recent study, Alekseev et al. prepared mixed complexes of β-lactam antibiotics (Ampicillin, Amoxicillin and Cephalexin) in solutions containing Co(II) and glycine anions(Gly). These complexes had been investigated using pH-metric titration at 20^{o}C in alkaline medium as mixed ligands complex [Co Gly Ampicillin], [Co Gly Amoxicillin], and [Co Gly Cephalexin] [9].

Instruments , Materials and Method

A - Instruments

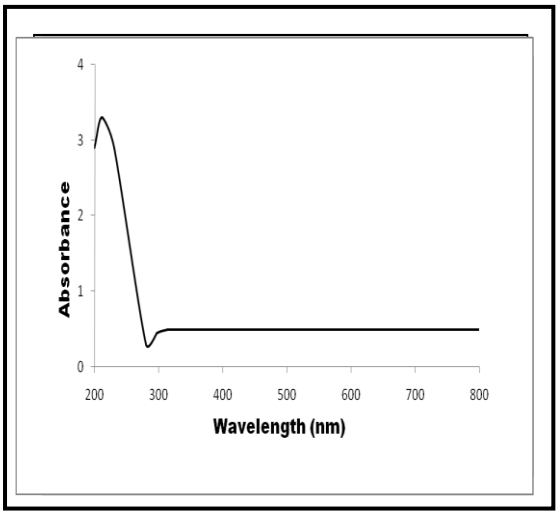
1. UV-Visible Spectrophotometer (CARY 100) wave length 200-1100nm.
2. Shimadzu (AA-670) Flame Atomic Absorption Spectrophotometer (400S).
3. Mettler, Balance Model 210S, ISO 9001.
4. pH-meter type 60A, USA.
5. Water Bath with Thermostat, Memmert.

B - materials

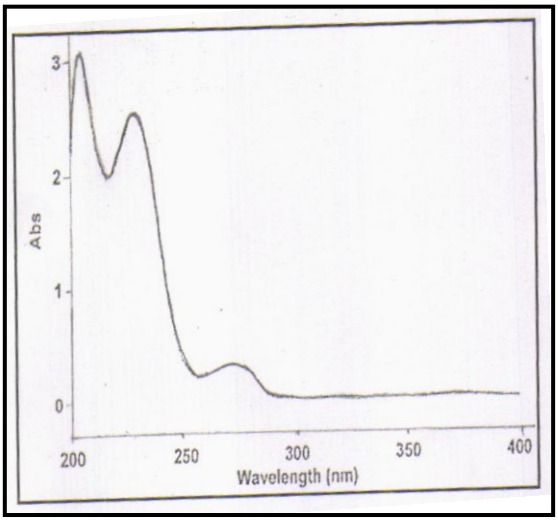
All the chemical stock solutions were prepared from analytical grade BDH, SDI, and India.

C- Method

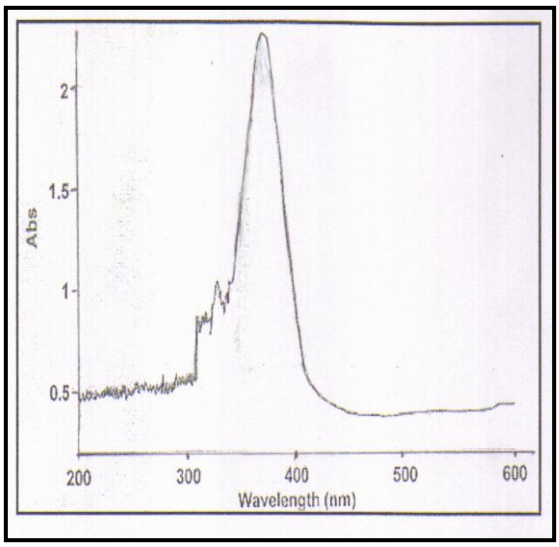
1. Stock solution of Co(II) 1000 ppm is prepared by dissolving 0.2010 gm hydrated cobalt chloride ($CoCl_2.6H_2O$) in distilled water and complete the volume to 50 ml.
2. Stock solution of Amoxicillin 1000 ppm is prepared by dissolving 0.1 gm amoxicillin in 5 ml. of 1M HCl then complete to 100 ml with distilled water.
3. Choosing the optimum conditions for complex formation: The experimental work showed that the reaction did not proceed at room temperature; heating was needed, media must be acidic for this reason. We studied the effect of pH, temperature, reaction time, extraction time, and suitable solvent for the extraction process.
4. Spectral study:
   - **a)** Amoxicillin spectrum: transfer 1 ml from stock solution of amoxicillin to 5 ml volumetric flask then dilute with distilled water the absorbance is measured at (200-1100 nm) using acidic water as blank. Table (2) shows the proper pH of the solution and Figure (1) shows UV spectrum for Amoxicillin.
   - **b)** Cobalt spectrum: transfer 1 ml of Co (II) stock solution to 5 ml volumetric flask then dilute with distilled water the absorbance is measured at (200-1100 nm) using distilled water as blank. Figure (2) shows UV spectrum for Cobalt.
   - **c)** Amoxicillin-Cobalt(II) [Amox.-Co(II)] Complex Spectrum: transfer (2-5 ml) from the standard of Amoxicillin stock solution to 5 ml volumetric flask then 1 ml of Cobalt stock solution is added. The chelating complex was extracted by 5 ml benzyl alcohol then measures the absorbance at (200-1100 nm) using benzyl alcohol as blank. Figure (3) Shows UV spectrum for [Amox.-Co (II)] Complex.



Figure(1): UV Spectrum of amoxicillin



Figure(2) :UV Spectrum of the element Co(II)



Figure(3): UV Spectrum of [Amox-Co(II)] Complex

Results and Discussion

Amoxicillin spectrum, illustrated at Figure (1), consist of 2 bands at $\lambda_{max.}$ (272 nm) and (228 nm), we depend on the band at $\lambda_{max.}$ (228 nm) because the other peak will disappear in some experiments also it may interfere with benzyl alcohol peak.

- Co (II) Spectrum gives peak at $\lambda_{max.}$ (211 nm) using distilled water as a blank, (Figure 2).
- Chelating complex [Amox.-Co (II)], a new peak at $\lambda_{max.}$ (375 nm) as shown in Figure (3) which indicate the formation of the complex that extracted by benzyl alcohol. Table (1) shows the color and $\lambda_{max.}$ for the amoxicillin, Co (II), and the complex.

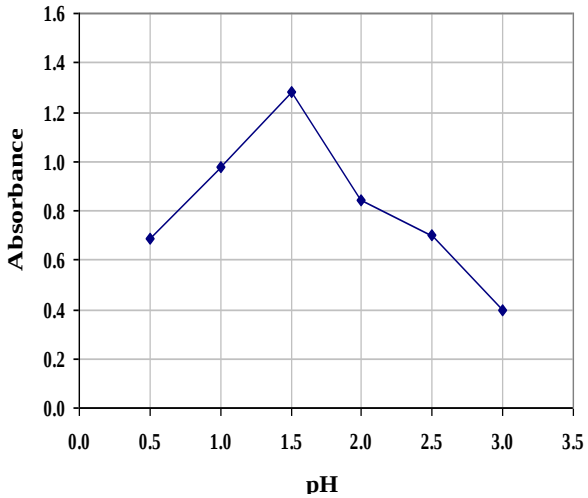
Compound	$\lambda_{max.}$ (nm)	Color
Amoxicillin	228 272	Off- white
Co(II)	211	Bluish- white
[Amox.-Co(II)]	375	White

Table (1): Color and λ_{max} for the drug, metal, complex.

- Detection the optimum conditions for complexation using UV-Visible Spectrophotometer:
  1. pH effect: Table (2) shows the absorbance of the [Amox.-Co (II)] complex using different pH (0.1-3), the optimum pH for the complexation was (1.5) where the absorbance is gradually increased from 0.5 to the maximum peak at pH 1.5 and then it decreases. Figure (4) shows the effect of pH on the absorbance of [Amox.-Co (II)].

Table (2): Complex absorption values at different pH.

pH	Absorbance
0.5	0.69
1.0	0.98
1.5	1.28
2	0.84
2.5	0.7
3.0	0.4

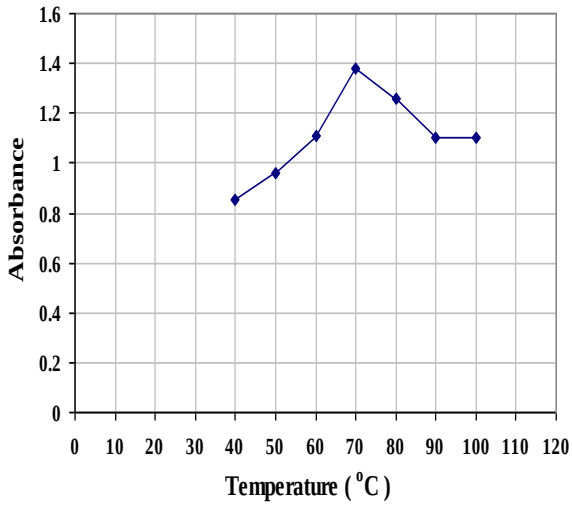


Figure(4): The effect of pH on the absorbance of the [Amox-Co(II)] Complex

2. Temperature effect: the reaction of the metal and Amoxicillin proceeded slowly, to increase the reaction velocity we intend to increase the temperature of the reaction from 40 to100 ˚C, then the complex is extracted and measured the absorbance using UV Spectrophotometer. Table (3) gives the absorbance of the complex at different temperatures at pH 1.5 and Figure (5) shows the λ_{max} for the complex is at 70 ˚C.

Table (3): Complex absorption values at different temperatures at (pH 1.5).

Temp. ^{o}C	Absorbance
40	0.85
50	0..96
60	1.11
70	1.38
80	1.26
90	1.1
100	1.1



Figure(5) The effect of temperature on the [Amox-co(II)] formation

3. Reaction time: the complex formation increased as well as the absorbance when the reaction time is increased, before the extraction process takes place, we used different times (5-30 min.), Table (4) and Figure (6) showed that 20 min. is the best time for the reaction and give maximum absorbance for the [Amox.-Co (II)] at (70 ˚C and pH 1.5).

Table (4): Complex absorbance value at different reaction time at 70 ^{o}C

Time(min.)	Absorbance
5	0.75
10	0.99
15	1.16
20	1.30
25	1.22
30	1.13

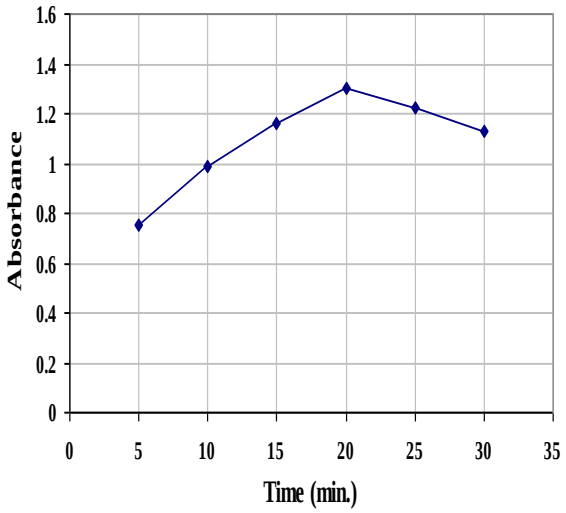


Figure (6) The effect of reaction time on complex formation

4. Suitable extraction solvent: different organic solvents were used like; methanol, ethanol, chloroform, benzyl alcohol, and ethyl acetate to choose the proper solvent that dissolves the complex, but can not dissolve the metal (Cobalt) and amoxicillin as well give the highest absorbance for the complex. Table (5) shows the solubility of the amoxicillin, metal and the complex in different organic solvents.

Table (5): The solubility of amoxicillin, metal and the complex in different solvents.

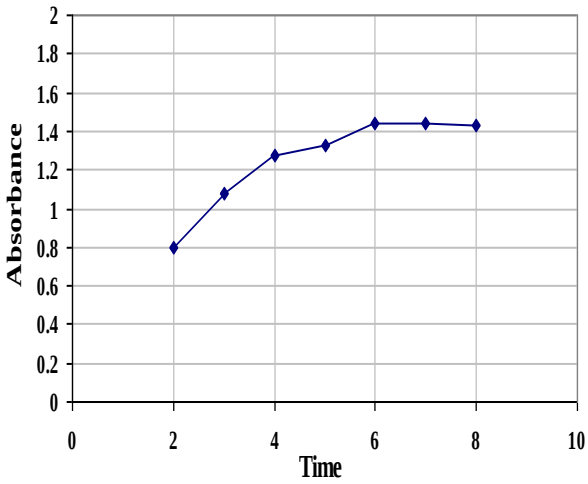
Solvent	Amoxicillin solubility	Metal solubility	Complex solubility
Methanol	+	+	–
Ethanol	+	+	–
Chloroform	–	–	–
Benzyl alcohol	–	–	+
Ethyl acetate	+	–	+

(+): Soluble.
(–) :Very slightly soluble or insoluble

5. Extraction time: the optimum time for shaking during the extraction of the complex was 6 min. which gives maximum absorbance as shown in Table (6) and Figure (7).

Table (6): Effect of shaking time on the extraction process against the absorbance.

Extraction time (min)	Absorbance
2	0.8
3	1.08
4	1.27
5	1.33
6	1.44
7	1.44
8	1.43

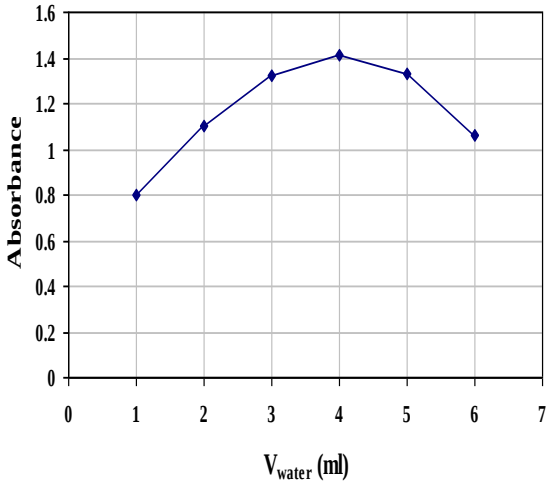


Figure(7) The effect of the extraction time on the absorbance of the complex

6. Solvent phase ratio: it was found that 4 ml of aqueous phase and 4 ml of organic phase is enough to give maximum absorption for the complex as shown in Figure (8). Table (7) showed that the increase in the aqueous phase to 6 ml with 4 ml of organic phase will decrease the absorbance of the complex.

Table (7): Absorbance value of the [Amox.-Co(II)] as the volume of water phase increased.
Note: organic phase volume = 4 ml.

Water phase volume (ml.)	Absorbance
1	0.8
2	1.1
3	1.32
4	1.41
5	1.33
6	1.06

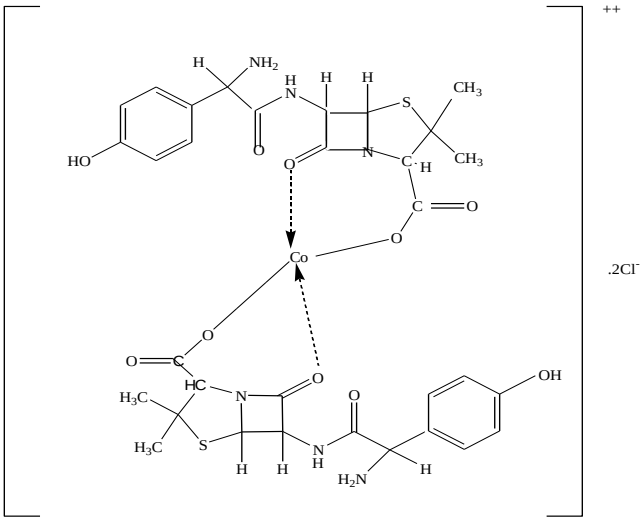


Figure(8): The effect of water volume on the absorbance of the [Amox.-Co(II)] Complex

7. Times of extraction: the first extraction process is enough to extract the major concentration of the complex because the second extraction process for the complex that remained in aqueous phase gives a very weak absorbance less than 0.1.

Calculation of the ligand (Amoxicillin) to metal (Cobalt) ratio in the complex [Amox.-Co (II)]:

Molar ratio: to detect the ratio of complexation i.e. the molar ratio of Ligand (L) to the Metal (M) by taking different volumes of the Ligand (V_L) with constant volume of Metal (V_m) at the same concentration for each (1.2×10^{-3} M) at the optimum conditions for complexation, then drawing the relation between absorbance and V_L/V_m as in Figure (9), and Table (8) shows the absorbance against V_L/ V_m. The molar ratio about (2:1) for the (L: M) and the suggested chemical structure for the [Amox.- Co(II)] complex is:



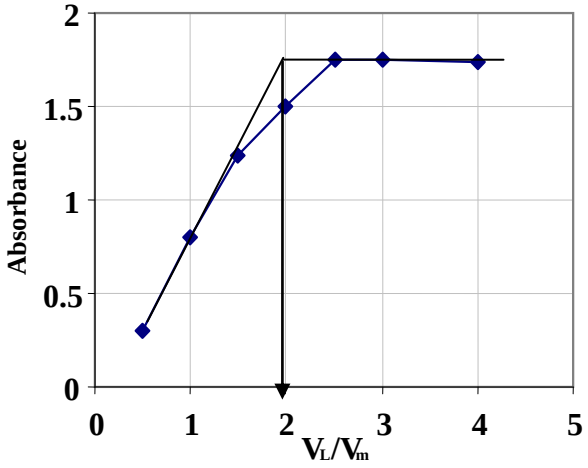
pH = 1.5

Co(II)+2Amox. ⟶ Co(II) – 2Amox.

Imran [3] and his partners defined the Amoxicillin complex form with transition elements in M:L is 1:2 ratio, and this was identical with proportion of metal with the ligand (drug) that we have reached by molar ratio.

Table (8): The values of the absorbance of the complex against V_L/V_m

V_L/V_m	Molar ratio	Absorbance
1/2	0.5	0.3
2/2	1	0.8
3/2	1.5	1.24
4/2	2	1.5
5/2	2.5	1.75
6/2	3	1.75
8/2	4	1.74



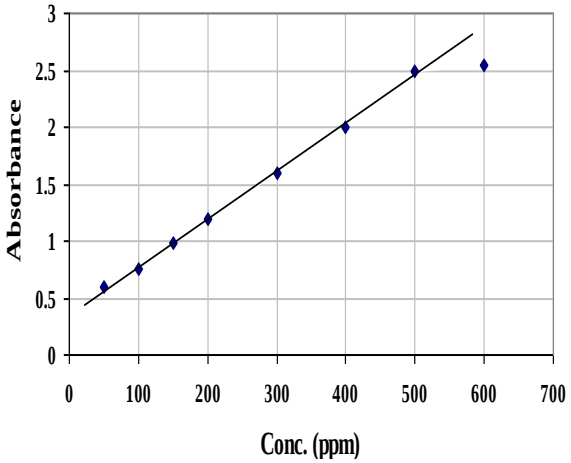
Figure(9) : Detection the molar ratio for the complex formation

Calibration curve of the UV spectrum:

It was carried out through taking different concentrations for the complex (ppm) and measuring the absorbance, as shown in Table (9). Figure (10) shows the calibration curve of [Amox.-Co(II)] complex that obeys Beer's law for the concentrations (50-500 µg. ml $^{-}$) at 375 nm.

Table (9): The value of maximum absorbance for the [Amox.-Co(II)] at different concentrations.

Conc. ppm	Absorbance
50	0.6
100	0.75
150	0.98
200	1.2
300	1.6
400	2.0
500	2.49
600	2.55



Figure(10): The calibration curve for detecting of [Amox.-Co(II)] at 375 nm

Determination of the drug concentration in different pharmaceutical preparations:

This was done by taking an average weight of one capsule from six capsules that have been mixed previously then the absorbance of the active ingredient (Amoxicillin) was measured from calibration curve (Figure 10), after complexation process takes place at the optimum condition mentioned above.

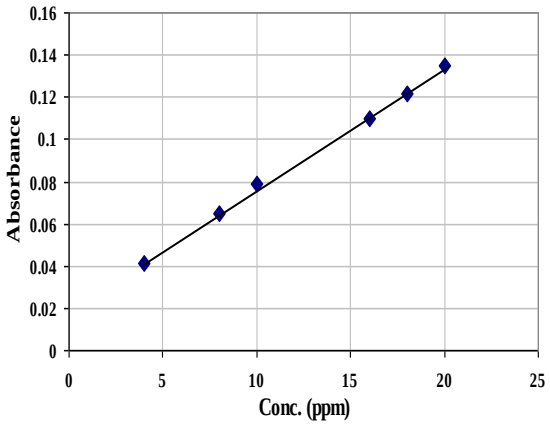
Note: we carried out the same procedure for 500 mg. amoxicillin capsules of different trademarks (SDI, Julphar and Ajanta). Table (10) shows the results of these preparations.

Table (10): The concentration of amoxicillin in 500 mg amoxicillin capsule of different trademarks.

Capsule	Absorbance	Conc. ppm
SDI	2.5	501
JulpHar	2.48	499
Ajanta	2.42	487

Determination of the [Amox.-Co(II)] by Flame Atomic Absorption Spectrophotometer (FAAS):

The complex was prepared under the optimum conditions of pH, temperature, proper solvent etc.. and we used the FAAS to detect the amoxicillin concentration by indirect measurement the absorbance of the Co(II) in the complex as shown in Figure (11), also we can measure the concentration of the amoxicillin in these pharmaceutical preparations using the calibration curve of indirect (FAAS).



Figure(11): The calibration curve for detecting amoxicillin using FAAS

Conclusion

The developments of new analytical methods to determine the amount of amoxicillin, such methods are UV-Visible and flame atomic absorption spectrophotometrics, are very sensitive and precise. Amoxicillin forms chelated complex with Cobalt ions at 70^{o}C and pH: 1.5 and the molar ratio for complex formation, Amoxicillin:Cobalt, is (1:2). The results of analysis of amoxicillin capsules of different trademarks show the amount of amoxicillin in SDI and Julphar capsules are almost as presented by the package, on the other hand the Ajanta capsules show some differences.

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Proceeding Bromometric Phenol Assay without Starch Indicator[#]

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Abstract

In this research, we exclude starch indicator preparation,that is used in official phenol assay method. The liberated iodine, in presence of chloroform, was acting as indicator and titrated with sodium thiosulfate until getting a sharp colorless end point. Similarly, starch was cancelled during both blank and standardization of bromine water solution experiments needed in phenol assay. The results obtained were the same volumes and weights as that achieved using starch with just about 0.03% difference in sample procedure. Finally, this work will enable us to save time, effort, fuel and materials spended in laboratory.

Key word:- Phenol, assay, starch indicator

الخلاصة

هذا البحث يقوم على الغاء تحضير محلول النشا المستعمل كدليل عند تحليل الفينول رسمياً حيث نقوم بتسحيح اليود بوجود الكلوروفورم والذي يعطينا لوناً احمر غامق مع محلول الثايوسلفات الصوديوم لنحصل في النهاية على محلول شفاف ونقطة نهاية واضحة بدون استعمال محلول النشا وينطبق هذا ايضاً على تجربتي ايجاد البلانك وتقييس محلول البروم اللتين نحتاجهما خلال عملية التحليل. ولقد اعطتنا هذه الطريقة المختصرةنتائج دقيقة بالنسبة لتجربة التقييس والبلانك مع فارق ضئيل يقدر بحوالي 0.03% لتجربة التحليل وبهذا العمل نستطيع توفير الوقت، الجهد، الطاقة والمواد المصروفة في المختبر.

Introduction

Phenol or so-called carbolic acid is a colorless to pale pink crystalline material with a characteristic medicinal odour. It is slightly soluble in water but freely soluble in some organic solvents and can be present as liquefied phenol. It is still used occasionally as an antipruritic in phenolated calamine lotion exerting local anesthetic effects. It remains the standard to which the activity of most germicidal substances is compared with phenol coefficient of 1.0 [(1)] Phenol is preserved in tight and light-resistant containers with suitable stabilizer [(2a)]. It is identified with ferric chloride or bromine solution [(3a)].Phenol can be thought of as hydroxy derivative of benzene. It occurs widely throughout nature mainly obtained from coal tar. It is a general disinfectant and it serves as intermediate in the industrial synthesis of products as diverse as adhesives and antiseptics. It was used for manufacturing the explosive picric acid. It can be used as Bakelite resin and adhesives for binding plywood. It is also the starting material for the synthesis of chlorinated phenols and the food preservative BHT (Butylated Hydroxytoluene) & BHA (Butylated Hydroxyanisole). Pentachlorophenol is widely used as wood preservative. The herbicide 2, 4-D(2, 4- dichlorophenoxy acetic acid and hospital antiseptic hexachlorophene are derivatives of phenol.Phenol is oxidized with strong oxidizing agents (like Fremy's salt) yielding a cyclohexa-2, 5-diene -1, 4-Dione (Quinone) [(4)].This oxidative dearomatization to quinones also known as the Teuber reaction using oxone as oxidizing reagent [(5)].phenol is also used in the preparation of cosmetics including sunscreens [(6)].Phenol can be made also by fusing sodium benzene sulfonate with NaOH or by heating mono chlorobenzene with aqueous NaOH under high pressure [(7)].Phenol may be formed endogenously from metabolism of other xenobiotics,notably benzene,and by catabolism of protein and other compounds by gut bacteria[(8)].Under laboratory conditions mimicking hydrothermal circulation (water,200^{o}C,1.9 GPa),phenol is found to form from sodium hydrogen carbonate and iron powder [(9)].The most striking chemical property of phenol is as extremely high reactivity of its ring toward electrophilic substitution as a strong ortho-and para-director potentiated with its acidity [(10)],and it has been recently shown that only about 1\3 of the increased acidity of phenol is due to inductive effects,with resonance accounting for the rest [(11)]. Phenol,as oily injection,is used to inject haemorrhoids particularly when unprolapsed [(12)].Simply heating a mixture of phenol and formaldehyde with aqueous acid leads Bakelite which was the first commercially available cross-linked three dimensional network polymer molecule that is very resistant to solvents, heat and electricity and widely used in household products [(13)].

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Phenol is incompatible with alkaline salts and nonionic surfactants. The antimicrobial activity of phenol may be diminished through increasing pH or through combination with blood and other organic matters. It should not be used to preserve preparations that are to be freeze-dried [14].A ten-day,nose-only,phenol inhalation toxicity study in Fischer 344 rats did not find evidence of adverse effects [15],in addition ,phenol has been evaluated in vivo studies using specialized protocols [16].The aromatic C-O bond is difficult to break in phenol using strong acids like HBr to form bromobenzene. Thus HBr can protonate phenol, but no further reaction occurs [17].Spore proteins of Aspergillus versicolor, as an indoor mould, can be purified using phenol extraction with subsequent solvent precipitation and washing steps. This protein was prepared for two-dimensional (2D)-gel electrophoresis with sera from patients to study about indoor exposure of moulds and their influence on the development of allergies by screeing sera for IgE antibodies specific for A. versicolor and others [18].Hydroquinone,as a member in phenols family,is assayed with volumetric titration unlike the bromometric method used for phenol [2b] while resorcinol assay followes the later one [3b].Phenol showes a characteristic broad IR absorption at 3500 Cm^{-1} due to the-OH group, as well as the usual 1500 and 1600 Cm^{-1} aromatic bands in addition to monosubstituted aromatic ring peaks at 690 and 760 Cm^{-1} while it possesses H NMR absorptions near 7-8 S of aromatic ring protons. Phenol-OH protons absorb at 3-8 S [4].These spectroscopies are used for identification of phenol together with ferric chloride or bromine solution chemical tests mentioned previously. Accordingly, phenol reaction with bromine gives 2, 4, 6-tribromophenol [19] as a white chloroform soluble precipitate and this is the principle of quantitative phenol assay that is officially followed in USP and B-P using starch as indicator. Therefore we are going to proceed the same procedure for assaying phenol with the exception of no need to add starch.

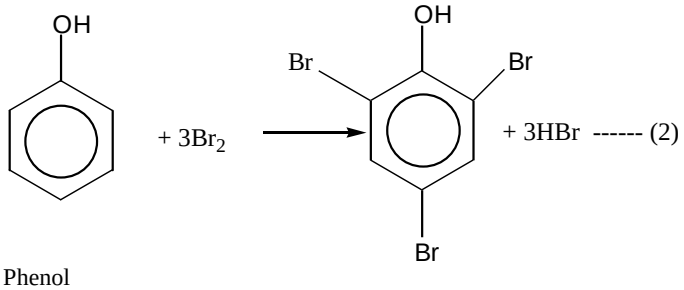
Materials and method

Potassium bromate from BD; Potassium bromide, M&B; Phenol, BDH; Sodium Thiosulfate, Hopkin & Williams Synchemica, England. And Chlorofrom, GCC, United Kingdom. England; Potassium iodide, Merck; hydro chloric acid, Riedel-De Haen; Starch, Merch, Germany;25 ml-bulb pipette,Din,West Germany;50 ml- Burette,Permagold,Exelo;500 ml-volumetric flask,pyrex,USA;500 ml-iodine flask,Schott(Witeg),West Germany.The method used for assaying phenol here is the same as that mentioned in USP or BP and it depends on oxidation-reduction reaction steps.First of all, we did standardization for bromine (bromide and bromate) solution by taking 25 ml into 500ml- iodine flask and 120 ml distilled water was added followed by 5 ml concentrated HCL, the flask was then stoppered and shaken gently, we added 5 ml of 20% potassium iodide solution and restoppered the flask. The mixture was shaken, allowed to stand for 5 min. and then titrated with 0.1N sodium thiosulfate using starch indicator which gave deep blue colour with liberated iodine. The end point is indicated with colorless solution. We repeated the standardization procedure but without starch indicator (here we can also add 5ml of chloroform to act as co-indicator with iodine) and recorded the volumes of sodium thiosulfate of both experiments. The equation $N_1V_1=N_2V_2$ is used to find the normality of bromine solution and the results are shown in table 1. These events are represented with equations 1, 3 & 4 [2c].At second stage, we did bromometric phenol assay which includes addition of excess (50 ml of 0.1N) bromine solution to 25 ml-phenol solution (sample1, which was prepared by dissolving 1.1gm phenol in 500 ml water to get 0.055gm /25 ml) and then liberation of bromine by addition of 5 ml concentrated HCl. Bromine reacts readily with phenol through electrophilic aromatic substitution yielding 2, 4, 6-tribromophenol as a white precipitate. Stoppering well the iodine flask is necessary to prevent escape of bromine vapour. The flask was shaken repeatedly for 30 min., leave to stand for 15min.and 5 ml of 20% potassium iodide solution was added with contineous shaking and the flask and stopper was washed with water and 5 ml chloroform was then added to dissolve the precipitate. Iodine, released due to potassium iodide reaction with bromine, was titrated against 0.1N sodium thiosulfate solution until pale yellow occurred. 1-2 ml starch solution was added giving deep blue colored complex with iodine and we continued the titration until discharging the colour to a clear colorless end point. Again, we repeated the same experiment above but without adding starch indicator and, here, the solution became brown-deep red colour due to the presence of iodine itself which was then titrated versus sodium thiosulfate solution until sharp colorless end point. In the same way, we repeated the same experiments (with and without starch) on second amount of phenol (sample 2), recorded the volumes of sodium thiosulfate needed for

each pair of samples knowing that each 1ml of 0.1 N bromine solution is equivalent to 0.001569 gm of phenol (the chemical factor, which is number of gm weight equivalent to 1 ml of standard solution) and the resultant weights were shown on tables 3 and 4. equations 1-4 are the principle of phenol assay.Finally, we had to do blank without phenol twicely (with and without starch too) and the volumes of titrant were recorded to be employed mathematically. The results were shown in table 2 . Equations 1,3 and 4 represented these reactions.

Note:- all the volumes, except that of standardization, of sodium thiosulfate must be corrected to 0.1 N.

$5KBr+KBrO_3+6HCl \rightarrow 3Br_2+6KCl+3H_2O$ ---------- (1)



Phenol

2,4,6-tribromophenol(White ppt.)

$Br_2 + 2KI \rightarrow I_2 + 2KBr$ -------- (3)

excess (unreacted)

$I_2 + 2Na_2S_2O_3 \rightarrow 2NaI + Na_2S_4O_6$ ------- (4)

Equation (4) represents the end point.

Scheme I: Sequential equations of phenol assay

Results and Discussion

As shown in tables 1,2, 3 and 4 we see the followings:-

- The volume of sodium thiosulfate solution for standardization of bromine solution with starch experiment was as exact as that without starch experiment and therefore the normalities of bromine solution for both experiments will be exactly the same.
- Therefore, total bromine solution that must be added is the same for blank and assay experiments with and without starch procedure.
- The volume of $Na_2S_2O_3$ of blank experiment with starch was nearly exact to that without starch experiment and therefore the blank, that must be used mathematically, was also nearly the same for both experiment (after correction).
- For sample 1 , the volume of $Na_2S_2O_3$ of assay experiment with starch was closely related to that of without starch procedure and, as a result, the weight of phenol with starch was closely the same as that of without starch experiment.
- For sample 2 , on the other hand, the volume of $Na_2S_2O_3$ for assaying phenol with starch was also approximately equal to that without starch and the corresponding weights of phenol were approximating too.

Therefore, we see a small difference ranging from 0.03-0.04% (as shown on table 5) between the resultant weights of phenol assay with and without starch procedure possibly due to simple error in technique (may occur in measuring phenol samples since capacity of bulb pipette is 25±0.03 ml). Starch makes a deep blue color complex with iodine and should be added when iodine is in a low concentration (near the end point) and when all iodine is depleted, the solution becomes colorless[19a]. While in our new experiment, this disadvantage will be abolished since there is no starch present but, instead, iodine in the presence of chloroform will act as indicator exhibiting a deep red colour and, at the end point, the mixture will be colorless too. This resembles ascorbic acid assay procedure that runs using chloroform-iodine as indicator[19b].At last, starch preparation needs weighing, adding water, boiling the solution, cooling and then filtration to be ready for use. So, our modified experiment will have the advantage of saving time, fuel, effort and materials owing to similarity in quantitative and qualitative results that were obtained.

Table (1):- The results of standardization experiment with and without starch.

Experiments	Method with starch	Method without starch
N. of $Na_2S_2O_3$ must be 0.1N. N. of $Na_2S_2O_3$ prepared & used was	0.131 N	The same
V. of $Na_2S_2O_3$ for standardization of bromine solution prepared and used was	21 ml	The same

Table (2):- The results of blank experiment with and without starch.

Experiments	Method with starch	Method without starch
N. of bromine solution prepared and used was	0.11 N	The same
Total bromine must be used was	45.45 ml of 0.11 N	The same
V. of $Na_2S_2O_3$ 0.131 N needed for blank was	39.2 ml	39.15 ml
V. of $Na_2S_2O_3$ 0.1 N (after correction) needed for blank experiment was	(V blank) 51.352 ml	(V blank) 51.287 ml

Table (3) :- The results of sample 1 experiment with and without starch.

Experiments	Method with starch	Method without starch
Sample 1:- V. of $Na_2S_2O_3$ 0.131 N react with excess bromine solution was	13.45 ml	13.6 ml
Sample 1:- V. of 0.1 N of $Na_2S_2O_3$ react with excess bromine solution (after correction)	17.620 ml (Vexcess)	17.816ml (Vexcess)
V blank – Vexcess=Vreact with phenol=	33.732 ml	33.471ml
Vreactwith phenol X Chemical factor = weight of phenol in sample1	0.0529 gm	0.0525 gm

Table (4) :- The results of sample 2 experiment with and without starch.

Experiments	Method with starch	Method without starch
Sample 2 :- V. of 0.131 N $Na_2S_2O_3$ react with excess bromine solution was	26.7 ml	26.5 ml
V. of 0.1 N $Na_2S_2O_3$ react with excess bromine solution (after correction) Vexcess was	34.977 ml (Vexcess)	34.715 ml (Vexcess)
Vblank – Vexcess= V react with phenol=	16.375 ml	16.572ml
Vreact with phenol X chemical factor (0.001569) = weight of phenol in sample 2	0.0257 gm	0.0260 gm

Table (5) :- Weight (gm) difference between the weight of phenol assayed with and that assayed without starch for the two phenol samples.

Phenol sample	Obtained phenol concentration (gm/25ml)		Gm weight difference between with and without starch results
	With starch	Without starch	
Sample 1	0.0529	0.0525	0.0004 (0.04%)
Sample 2	0.0257	0.0260	0.0003 (0.03%)

Note:- Chemical factor:- 1 ml 0.1 N bromine=~ 0.001569 gm phenol

Conclusion

From this research we conclude that starch indicator preparation and addition can be no further continued whether at bromometric assay of phenol or at any iodometric titration with sodium thiosulfate as in ferric chloride colorimetric solution [(2d)].

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Effects of Different Concentrations of Melatonin on the Time-course of Nitrite–induced Oxidation of Hemoglobin: *In vitro* Study[#]

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Abstract

Melatonin is a potent scavenger of reactive oxygen species or free radicals like superoxide and hydroxyl radicals. The oxidation of hemoglobin to methemoglobin (meth-Hb) by oxidizing compounds has been widely studied. The present work was designed to evaluate the ability of different concentrations of melatonin to inhibit nitrite–induced oxidation of hemoglobin. Blood samples were obtained from apparently healthy individuals from which erythrocyte hemolysate was prepared. Different concentrations of melatonin (10^{-9}-1.0 mg/ml) were incubated for 10 min with the hemolysate, then to the resultant mixture 1 ml of sodium nitrite (final concentration 0.6 mM) was added, and the formation of meth-Hb was measured by monitoring absorbance of light at 631 nm each min for 30 min. Control samples without melatonin were utilized for comparison. Nitrite caused rapid oxidation of hemoglobin to meth-Hb in control samples; in the presence of melatonin, the oxidation process was delayed in a dose–dependent manner. The effect of melatonin on the time course of nitrite-induced oxidation of Hb showed that melatonin has a protective effect initiated early after addition along with nitrite. Melatonin also affect the time required for the formation of meth-Hb, the time required to convert 50% of the available Hb to meth-Hb was 4 min in the absence of melatonin, and became 17, 22, 26, 30, 114 and 383 min with increasing melatonin concentrations (10^{-9}, 10^{-6}, 0.001, 0.01, 0.1, and 1.0 mg/ml respectively). In conclusion, melatonin in a concentration and time dependent manner can protect Hb from oxidation by nitrite; melatonin delays the onset of autocatalytic stage and the protective effect extended over long period of time.

***Key words*: melatonin, erythrocytes oxidation**

الخلاصة

ان عملية اكسدة الهيمغلوبين وتحوله الى ميتهيمغلوبين قد تمت دراستها بشكل واسع، وتهدف الدراسة الحالية الى تقييم مقدرة تراكيز مختلفة من مادة الميلاتونين على منع أو تأخير حدوث عملية الأكسدة بمادة نايترايت الصوديوم. تم الحصول على عينات دم من أشخاص أصحاء وتحضير محلول من الهيمغلوبين من الكريات المتحللة وحسب الطرق المعتمدة من قبل الآخرين. تم مزج محلول الهيمغلوبين مع تراكيز مختلفة من مادة الميلاتونين (1-10^{-9} ملغم/مل) لمدة 10 دقائق تم بعدها اضافة مللتر واحد من مادة نايترات الصوديوم كعامل مؤكسد. تمت متابعة عملية التأكسد من خلال قياس مستوى الميتهيمغلوبين المتكون كل دقيقة باستخدام مطياف الأشعة فوق البنفسجية. أظهرت النتائج ان للميلاتونين القدرة على تأخير تأكسد الهيمغلوبين بصورة تعتمد على التركيز وفترة الخلط. ويمكن الأستنتاج بان الميلاتونين بأمكانه حماية الهيمغلوبين من التأكسد بواسطة نايترات الصوديوم وبصورة تعتمد على التركيز وفترة المزج.

Introduction

Recently, many experimental data provided unequivocal evidence about the formation and role of free radicals in biological systems. [(1)] Such reactive species may bring about oxidative damage to virtually all cell compartments, eventually leading to various pathologies and aging. [(2)] These studies prompted research on physiological antioxidant systems and molecules, and stimulated the development of natural or synthetic compounds that prevent oxidative stress and damage mediated by an enhanced formation of free radicals. [(3)] After the discovery of its radical-scavenging properties, melatonin (N-cetyl-5-methoxytryptamine) has been considered as a putative biological antioxidant but it has been questioned to whether it may have a real antioxidant function under physiological conditions; [(4)] its molecular mechanisms of action remain to be clarified. Interactions of melatonin contributing to its antioxidant effects *in vivo* may be lost during *in vitro* experiments; when it behaves *in vitro* as an electron donor, many electrophilic compounds, such as the hydroxyl radical, Fe^{+3}, or carbon centered radicals may act as acceptors in one-electron transfer reactions, which convert the indolamine to the indolyl cation radical. [(5)] Reactivity of melatonin with oxygen centered radicals, such as peroxyl or alkoxyl radicals, as well as a moderate activity towards lipoperoxyl radicals, has also been demonstrated.

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Although the exact relationship between such activity and the concentrations required to perform it is not clarified, its ability to scavenge a broad spectrum of radicals could allow melatonin to behave as an antioxidant in various and possibly complex ways. [6] This study was designed to investigate the antioxidant activity of melatonin in different concentrations using an *in vitro* model of nitrite-induced hemoglobin oxidation.

Material and method

Blood samples were obtained from apparently healthy individuals, and were centrifuged at 2500 rpm and 4°C for 10 min to remove plasma and the buffy coat of white cells. The erythrocytes obtained were washed thrice with phosphate-buffered saline and lased by suspending in 20 volumes of 20mM phosphate buffer pH 7.4 to yield the required hemolysate concentration of 1:20. Different concentrations of melatonin were incubated for 10 min with the hemolysate starting with stock solution (melatonin 1mg/ml) from which serial dilutions were made to give concentrations of 0.1, 0.01, 0.001, 10^{-6} and 10^{-9} mg/ml melatonin solution. Then to these incubated mixtures 1ml of sodium nitrite (final concentration 0.6 mM) were added and the formation of methemoglobin was measured by monitoring absorbance at 631 nm each min for 30 min using a spectrophotometer. [7] In the second part of the study, melatonin was added either before or at various time intervals (5 min and 10 min) after the addition of sodium nitrite to the hemolysate solution, and the formation of methhemoglobin was measured by monitoring the absorbance of light at 631 nm, and the results were compared with control samples without melatonin; all experiments were performed in triplicate and repeated many times.

Results

Nitrite causes a rapid oxidation of hemoglobin to methemoglobin, as shown in control curve (figure 1). In the presence of melatonin, the oxidation process was delayed in a dose-dependent manner. Figure1 describes the effect of different melatonin concentrations on the time- course of nitrite oxidation of hemoglobin; without melatonin, the time-course of oxidation shows a characteristic pattern of slow initial transformation followed by a rapid autocatalytic process; in presence of melatonin there is slow increase in absorbance related to reduced levels of methemoglobin formation in all test samples. Figure 2 showed that addition of melatonin to the incubation mixture, at different time intervals (after 5 and 10 min) during the autocatalytic phase, did not affect its ability to decrease meth-Hb formation. The time required to convert 50% of the available hemoglobin to met hemoglobin was (4 min) in the absence of melatonin, whereas with 1 mg/ml melatonin solution the time was increased to 383 min (6.4 hr) (table 1).

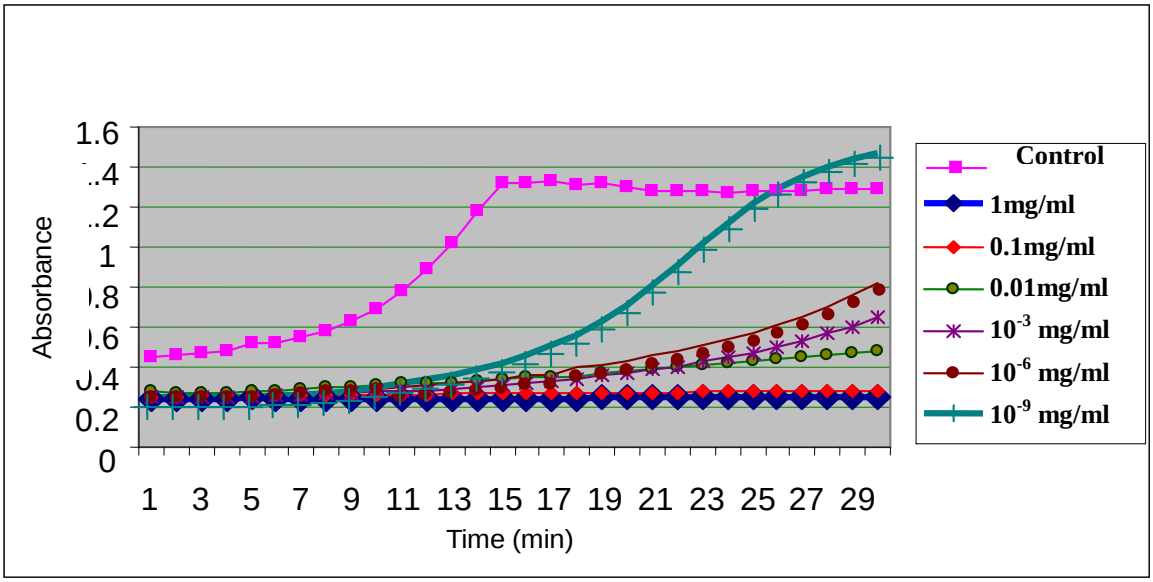

Figure 1. Effect of different melatonin concentrations on the time-course of nitrite–induced oxidation of hemoglobin.

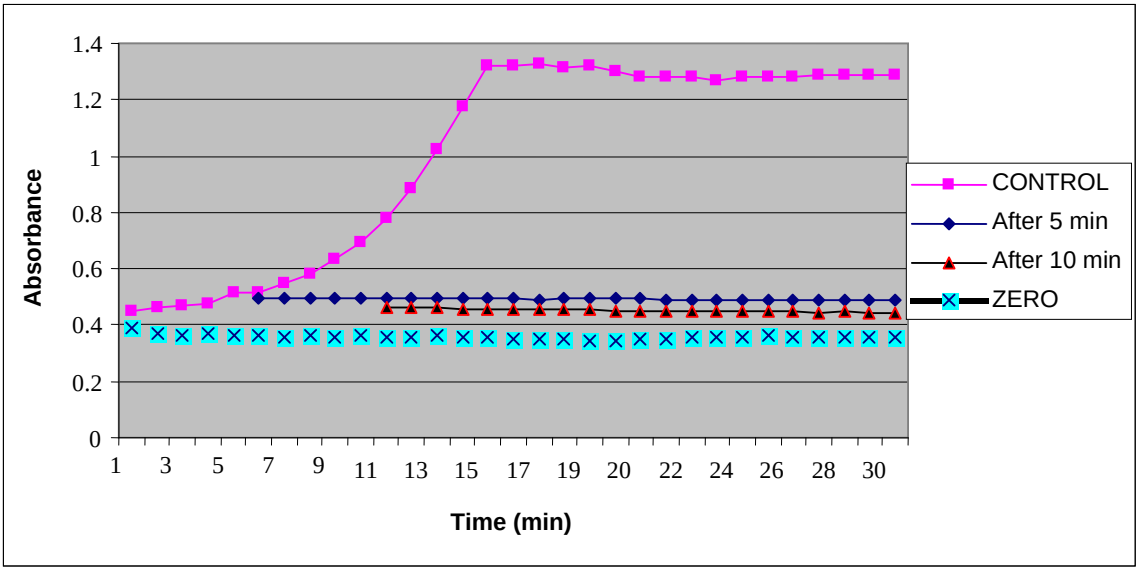

Figure 2. Effect of melatonin on the time course of methhemoglobin formation at various time intervals from nitrite addition.

Table(1) : Time to form 50% Meth-Hb in presence of different concentrations of melatonin.

Melatonin concentration mg/ml	% formation of Meth-Hb	Time to form 50% Meth-Hb (min)
Control	100	4.0
10^{-9} mg/ml	96.1	17.0
10-6 mg/ml	86.9	22.0
0.001 mg/ml	50.5	26.0
0.01 mg/ml	37.9	30.0
0.1 mg/ml	29.9	114.3
1.0 mg/ml	16.6	383.0

Discussion

The oxidation of Hb to Meth-Hb by nitrite has been widely studied, [7-9] formation of Meth-Hb occurs in two stages; there is a slow initial stage followed by a rapid autocatalytic stage, which carries the reaction to completion. [10] The present study has shown that melatonin can protect hemoglobin from oxidation by sodium nitrite in hemolysate, and there are two suggested theories for the mechanism through which melatonin produces this protective role; erythrocytes are utilized as a traditional target for studying oxidative damage, when exposed to high oxygen tensions and in presence of high iron contents (transition metal promoting the formation of oxygen free radicals) oxidative damage occur due to both endogenous and exogenous insults. Sodium nitrite as a prooxidant induces a primary extensive methemoglobin formation as a result of generation of several free radical species like super oxide anion, peroxynitrite, and nitric dioxide, which are generated during the course of nitrite–induced oxidation of hemoglobin. [11] After the discovery of radical-scavenging properties of melatonin, it has been considered a putative biological antioxidant, but it has been questioned whether it may have a real antioxidant function under physiological *in vivo* conditions. [6] The molecular mechanisms of actions of melatonin remain to be better clarified; it is capable to prevent the onset of the autocatalytic stage since superoxide is implicated in the autocatalytic stage , and the fact that melatonin is a potent scavenger of superoxide anion, [5] the results of the present study suggests that the protective action of melatonin might be due to its scavenger effect and not due to reduction of methemoglobin to hemoglobin, since it fails to reverse the oxidation of hemoglobin; additionally, direct interaction between nitrite and melatonin as a reason for protection can be ruled out because the concentrations of melatonin which protect erythrocytes is very low. [11] Kinetic evidence indicates that melatonin delays oxidative denaturation of Hb through it's reaction with Hb-derived oxoferryl radicals, and this may

explain the reported antioxidant effects; Tesoriere *et al* (2001) studied the reaction of melatonin with hemoglobin-derived oxoferryl radicals and the inhibition the oxidant effects of hydroxyl peroxide-induced hemoglobin denaturation in red blood cells, they found that the basic requirement for oxidative denaturation of Hb by hydroperoxides is the transient formation of the perferryl-Hb; [12] perferryl-Hb, which includes a hypervalent-iron oxoferryl heme group and a radical species, localized in the globin is a strong oxidant towards the globin moiety, which leads to Hb denaturation with the formation of hemichrome and heme release. [13] The perferryl species, generated from met-Hb and H_2O_2, [14] comprises a radical localized on the globin, possibly an aromatic amino acid radical , and an oxoferryl heme group. [12] After exhaustion of H_2O_2, decay of the perferryl to the oxoferryl form occurs, and then the latter is slowly converted to met-Hb by a so-called autoreduction reaction; [14] this process involves intramolecular electron transfer and modification of the globin moiety. [13] Such oxidative modifications of globin on exposure to H_2O_2 may be avoided by the presence of certain antioxidant compounds such as melatonin, ascorbate or Trolox at the time of reaction, suggesting that rapid deactivation of the protein radical in the perferryl species is crucial for protection; [15] the mentioned mechanisms may prove that melatonin acts through its reducing activity towards perferryl-Hb, and this may include the reduction of the oxoferryl moiety or the unpaired electron electrophile center at the globin moiety by melatonin, or both. [11] Although in our study we did not investigate the reactivity of melatonin as a reducing agent, we can not exclude this effect and it needs further investigations. In conclusion, melatonin protects hemoglobin against nitrite-induced oxidation and delay the formation of meth-Hb in concentration dependent pattern.

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