Vitamin C, Omega3 and paracetamol pharmacokinetics interactions using saliva specimen as determinater

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1.Introduction:

1.1.Paracetamol

Paracetamol (acetaminophen) is one of the most popular and widely used drugs for the treatment of pain and fever. It occupies a unique position among analgesic drugs.[1]It is available without a prescription in mono- and multi-component preparations, recommended for children and adults. The growing problem of safety of paracetamol questions the validity of easy access to the drug occurring in being-in-sale preparations with different names, dosages, forms and various ingredients combinations.[2]

Paracetamol is rapidly and almost completely absorbed from the gastro-intestinal tract. Paracetamol exhibits dose-dependent kinetics (first-order rate constant). Peak plasma concentration of paracetamol is usually reached within 30 min - 4 hours post ingestion with volume of distribution approximately 0.75-1 l/kg. About 90% of paracetamol is biotransformed by cytochrome P-450 in the liver as figure (1). Main metabolites are sulphate (about 52%) and glucuronide (about 42%) conjugates. About 4% of the drug is biotransformed to N-acetyl-p-benzoquinoneimine (NAPQI), which is a highly reactive cytotoxic intermediate. NAPQI is detoxified by conjugation with glutathione and excreted in the urine as cysteine and mercapturic acid. 90-100% of an ingested dose is excreted in urine during about 24 h [3 & 4] with about 4, 55, 30, 4 and 4% appearing as unchanged paracetamol and its glucuronide, sulphate, mercapturic acid and cysteine conjugates, respectively.[5]

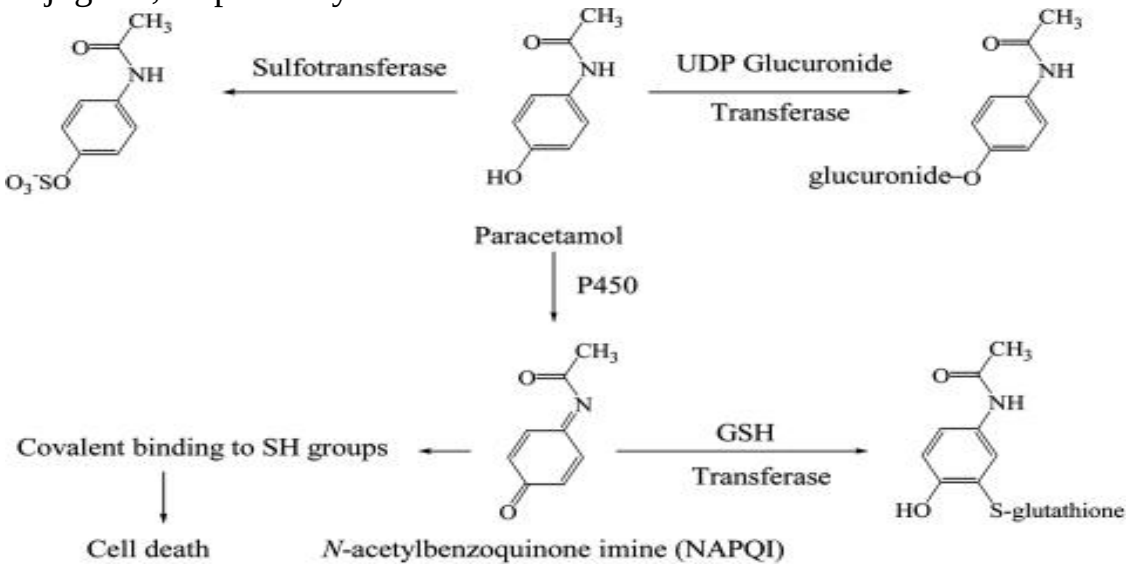


Figure 1:Metabolism of paracetamol in the liver.

Paracetamol rate of oral absorption is predominantly dependent on the rate of gastric emptying, being delayed by food, propantheline, pethidine and diamorphine and enhanced by metoclopramide. Age has little effect on the plasma half-life, which is shortened in patients taking anticonvulsants. The plasma half-life is usually normal in patients with mild chronic liver disease, but is prolonged in those with decompensated liver disease.[5]

1.2.Vitamin C:

Vitamin C (ascorbic acid)is a six-carbon compound structurally related to glucose. It consists of two inter-convertible compounds: L-ascorbic acid, which is a strong reducing agent, and its oxidized derivative, L-dehydroascorbic acid as figure (2).[6]

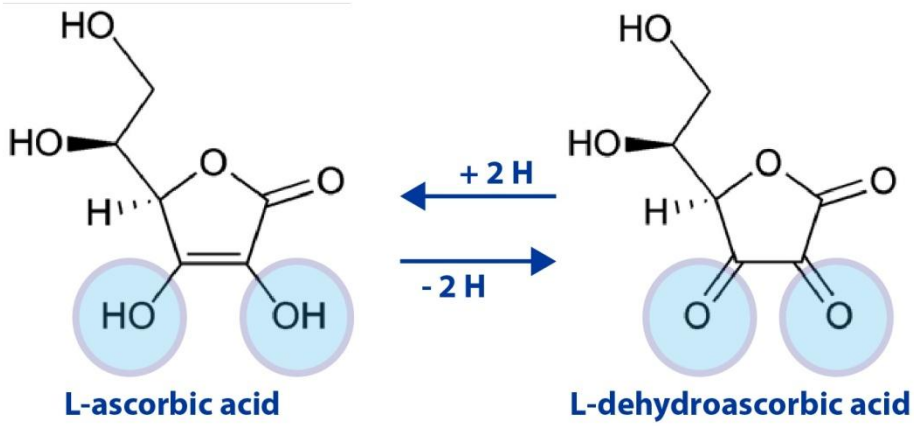


Figure 2: Metabolite of vitamin c (oxidation-reduction system).

Vitamin C is one of the important water-soluble vitamin which is important for lower blood cholesterol and contributes to the synthesis of the amino acids carnitine and catecholamine that regulate nervous system. It is needed for tissue growth and wound healing. It helps in the formation of neurotransmitters and increases the absorption of iron in the gut. Being an antioxidant, it protects the body from the harmful effects of free radicals and pollutants. Also vitamin C is used in the treatment and prevention of large number of disorders like diabetes, cataracts, glaucoma, macular degeneration, atherosclerosis, stroke, heart diseases and cancer. The intestinal absorption of vitamin C is regulated by at least one specific dose-dependent, active transporter and approximately 70%–90% of vitamin C is absorbed at moderate intakes of 30–180 mg/day. However, at doses above 1 g/day, absorption falls to less than 50% and absorbed, unmetabolized ascorbic acid is excreted in the urine.[8] Vitamin C is known to act as antioxidant

both in vitro and in vivo. Vitamin C, as an antioxidant agent, may have inhibited the chain reactions of acetaminophen-generated free radicals or scavenged the reactive free radicals before reaching their hepatic targets.[9] Also the property of vitamin C is an essential nutrient that functions as a non-enzymatic antioxidant in the cytosol that may affect on the metabolism of paracetamol.[10]

1.3.Omega 3:

Omega-3 fatty acids also called n-3 fatty acids which are polyunsaturated fatty acids (PUFAs) with a double bond (C=C) at the third carbon atom from the end of the carbon chain.[11] About **96%** of dietary Alpha-linolenic acid (ALA) appears to be absorbed in the gut. After absorption, ALA has several metabolic fates: 1) It can undergo oxidation to produce energy, 2) It can be recycled to make other fatty acids, 3) It can serve as a substrate for ketogenesis, the process of making ketone bodies, 4) It can be stored in adipose tissue for later use, 5) It can be incorporated into the phospholipids of cell membranes, where it affects membrane activities and 6) It can be converted tolong-chain omega-3 fatty acids like eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) as figure 3, which have important functions in many types of cells and organs.[12]

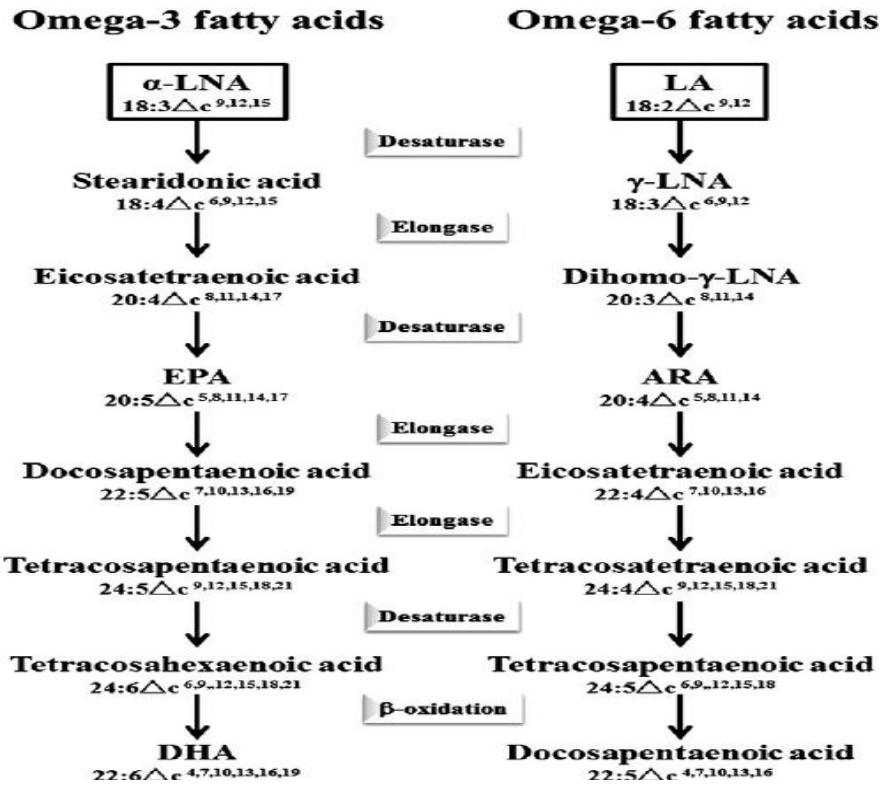


Figure 3: Metabolism of omega 3 and omega 6 fatty acids.

Omega 3 had cardio-protective effects such as antihyperlipidemia, antithrombotic, antiinflammatory, antihypertensive, and antiarrhythmic and anticancer effects.[13 & 14] omega-3 fatty acids from fish has antioxidant and antiradical activity.[15]antioxidants are substances that have the ability to delay or inhibit oxidation processes, even when used in small amounts, reducing the reaction rate or extending its period of induction. The effectiveness of an antioxidant is directly linked to increasing or prolonging the induction period of oxidation reactions of a substrate and can be expressed as an antioxidant index or protection factor, In the presence of antioxidants, the oxidative rates of glutathione decrease due to an increased activation energy for reaction, thus increasing the "lifetime" of the substrate.[16] Also omega 3 has membrane stabilizing as well as its conversion to lipid protective mediators in hepatocyte.[17] All these activities of omega 3 may be affected on paracetamol metabolism.

1.4. The aim of study:

The aim of our study is to estimate the effects of vitamin c and omega 3 (antioxidant agents) on the kinetics of paracetamol at different times.

2-Materials and methods:

2.1. Subjects:

Sex subjects (3male and 3 female) with mean of age (29) years, participate in the present study, which are taking 1000 mg single dose of paracetamol orally, then after a washout period again single dose of 1000mg of paracetamol plus 240 mg of vitamin C orally administered. After second washout period, they are taking 1000mg of paracetamol plus1000 mg of Omega3orally.

2.2. Exclusion criteria:

We exclude subjects with other diseases like liver disease, hypertension, diabetic mellitus, renal disease, endocrine problems and elderly.

2.3. Questionnaire form

We take the information from subjects about age, gender, weight, medical history in questionnaire form

Questionnaire form

Name of subject:

Age:

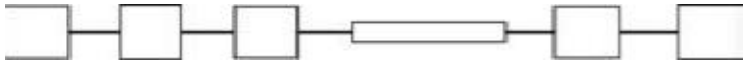
Gender:

Weight:

Medical history:

2.4.HPLC method:

High-performance liquid chromatography (HPLC) is a type of liquid chromatography used to separate and quantify compounds that have been dissolved in solution. HPLC is used to determine the amount of a specific compound in a solution.[18] The components themselves are first dissolved in a solvent and then forced to flow (via the mobile phase) through a column (stationary phase) under highpressure as figure (4 and 5).[19]



Mobile Phase Reservoir **Pump** **Injector for sample** **Column** **Detector** **Data display**

Figure 4. Schematic of a High-Performance Liquid Chromatograph (HPLC).[18]

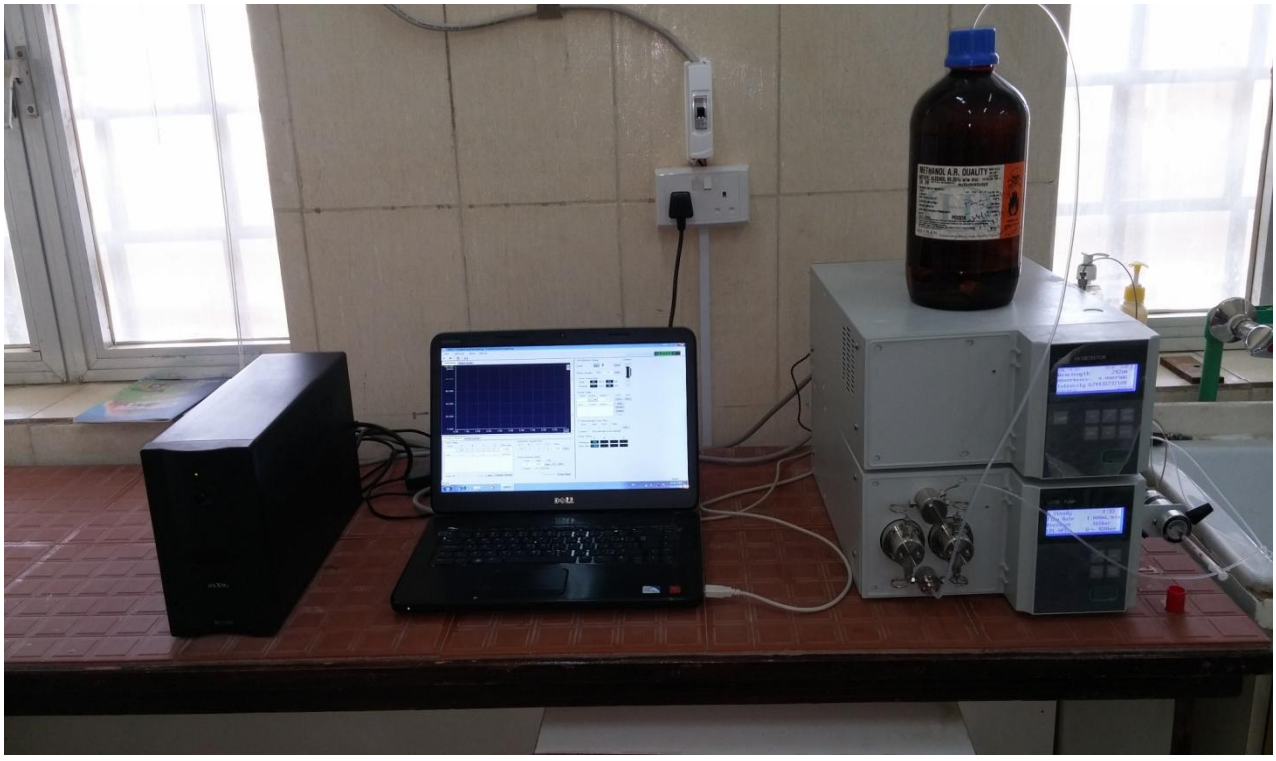


Figure 5: High-performance liquid chromatography (HPLC)

2.5.Mobile phase preparation:

Mobile phase was prepared from acetonitrile: methanol: water (12:12:76).. Solvents must be filtered to remove any particulate matter and degassed before use on an HPLC. prepare 1 L of a 12:12:76 acetonitrile:methanol:water solution and then filter this mixture through a nylon filter. The mobile phase can be sonicated for 20 minutes prior to use to remove dissolved air. mobile phase can be pumped through the column at a flow rate of 1.5–2.0 mL/min for about 20 minutes and then a mobile phase can be pumped at a flow rate of 2.0 mL/min for 20 minutes to equilibrate the column.[19]

2.6.Calibration curve:

The calibration curve was prepared by using five standard pointes. A stock solution of 10 mg/ml was prepared by weighing 10 mg of paracetamol precisely into a 100-ml volumetric flask and dissolved in methanol. Then, further four standard dilutions were made by frequent dilutions of 100 mg into 80,40,20,10 mg. Methanol was used for dilution using 10 ml volumetric flask.

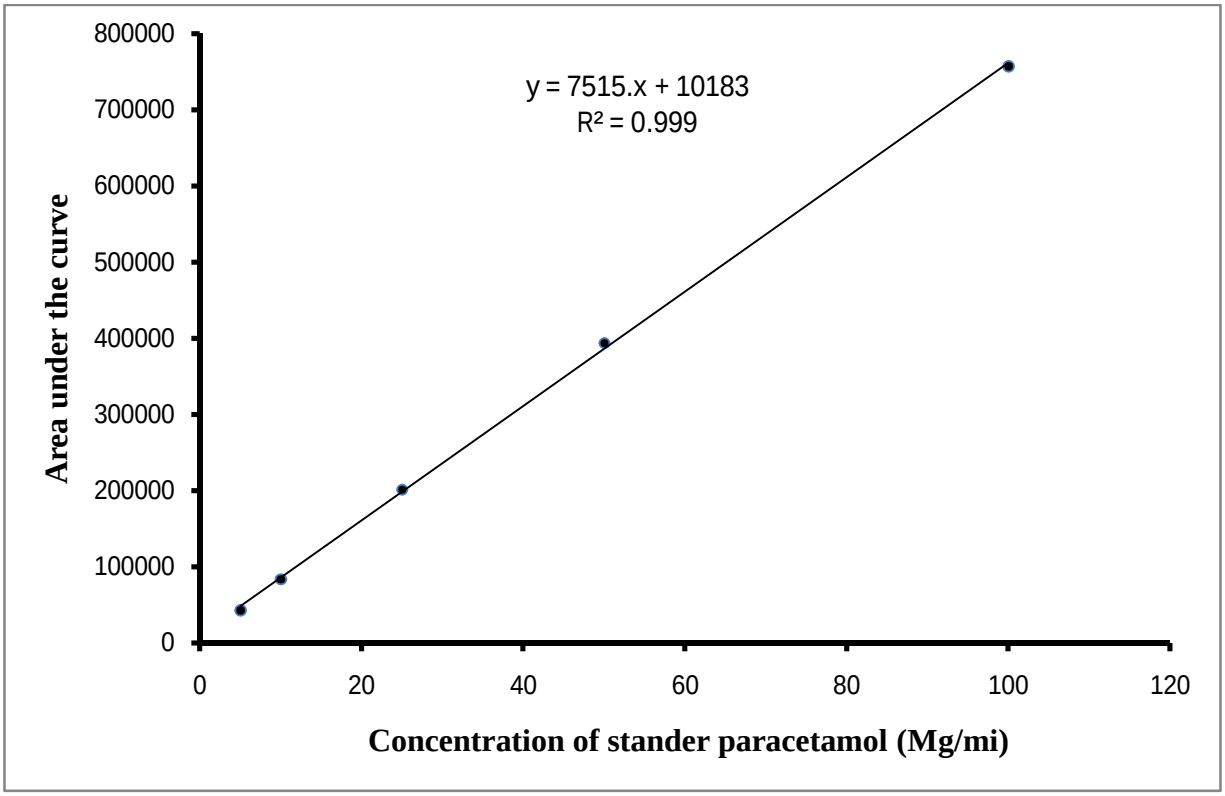


Figure 6: Paracetamol Calibration Curve

2.7. Procedure:

The use of saliva for measuring the secretion rate of paracetamol due to saliva sampling is less discomforting and risky than blood sampling. Also paracetamol has been found to pass readily from blood to saliva via the salivary glands resulting in similar concentrations in adult volunteers.[20]

1-Saliva samples are collect from subjects before and after they taking paracetamol.

2-200 Ml of acetonitrile add to 200 Ml of saliva (1:1) and mix them by rotary mixer.

3-Supernatant spread by using centrifuge (3000 rbm) for 10 min.

4-AUCs are measure for samples by HPLC and the concentrations of them determine by stander curve.

2.8. Statisttical analysis:

The data was analysed by SPSS version 15.The *t*-test was used to find the relationship between the concentrations of paracetamol in saliva with the time, also this test was used to find the relation between the concentrations of paracetamol plus vitamin C and Omega with the time.

3. Results:

The relationship between the concentration of paracetamol and time was significant at (1, 1.5, 2,2.5 hrs), where p values were (<0.05, 0.01, 0.01 and 0.01) respectively that mean the time factor may be affected on the absorption and metabolism of paracetamol as shown in table (1) and figures (7 &8).

After washout period, Subjects were taking paracetamol plus Vitamin c. The concentration of paracetamol plus vitamin c was a significant increase at first half hours (p <0.01). Also the concentrations of paracetamol plus vitamin c were significant with time at (1, 1.5, 2 hrs), where p values were (<0.01, 0.05, 0.01) respectively as shown in table (2) and figure (9).

After second washout period, the relationship between the concentration of paracetamol plus omega 3 with the time was significant for all times, where p values were (< 0.05, 0.05,0.05, 0.01 and 0.01) respectively as shown in table (3) and figure (10).

The relationship between the concentration of paracetamol, paracetamol plus vitamin c and paracetamol plus omega 3 was significant with time at (1, 1.5, 2,2.5 hrs), where p values were (<0.05, 0.01, 0.01 and 0.01) respectively as shown in table (4) and figure (11).

Table 1: Means, stander deviations and t-test for five groups which are taking paracetamol (1000 mg) only

Groups according to the sampling time	Mean	Stander deviation	*t*-test	P value
Group (1) After half hours N=6	2.6352	2.5237	2.558	0.051
Group (2) After one hours N=6	2.9638	2.0529	3.536	<0.05*
Group (3) After one and half hours N=6	2.7688	1.6201	4.186	<0.01 *
Group (4) After two hours N=6	2.2508	0.8347	6.605	<0.01 *
Group (5) After two and half hours N=6	2.1433	1.0303	5.096	<0.01 *

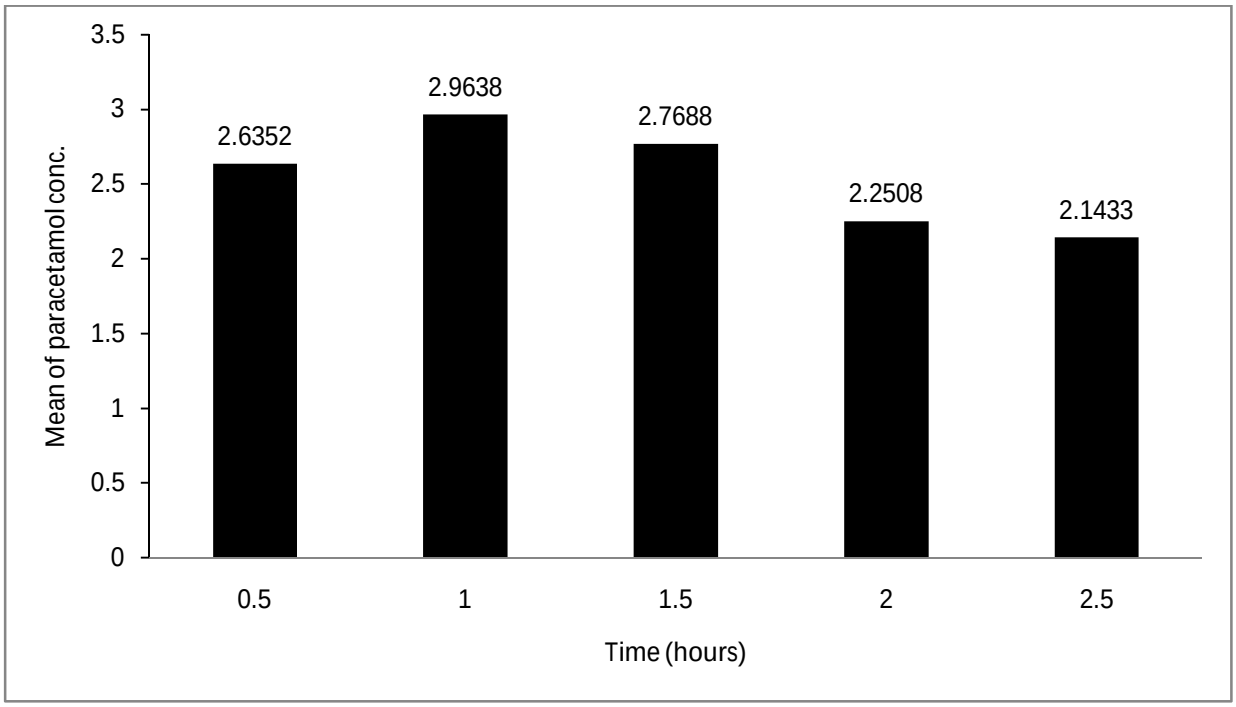


Figure 7:The relationship between the concentration of paracetamol with time at ½, 1, 1½, 2, 2½ hrs.

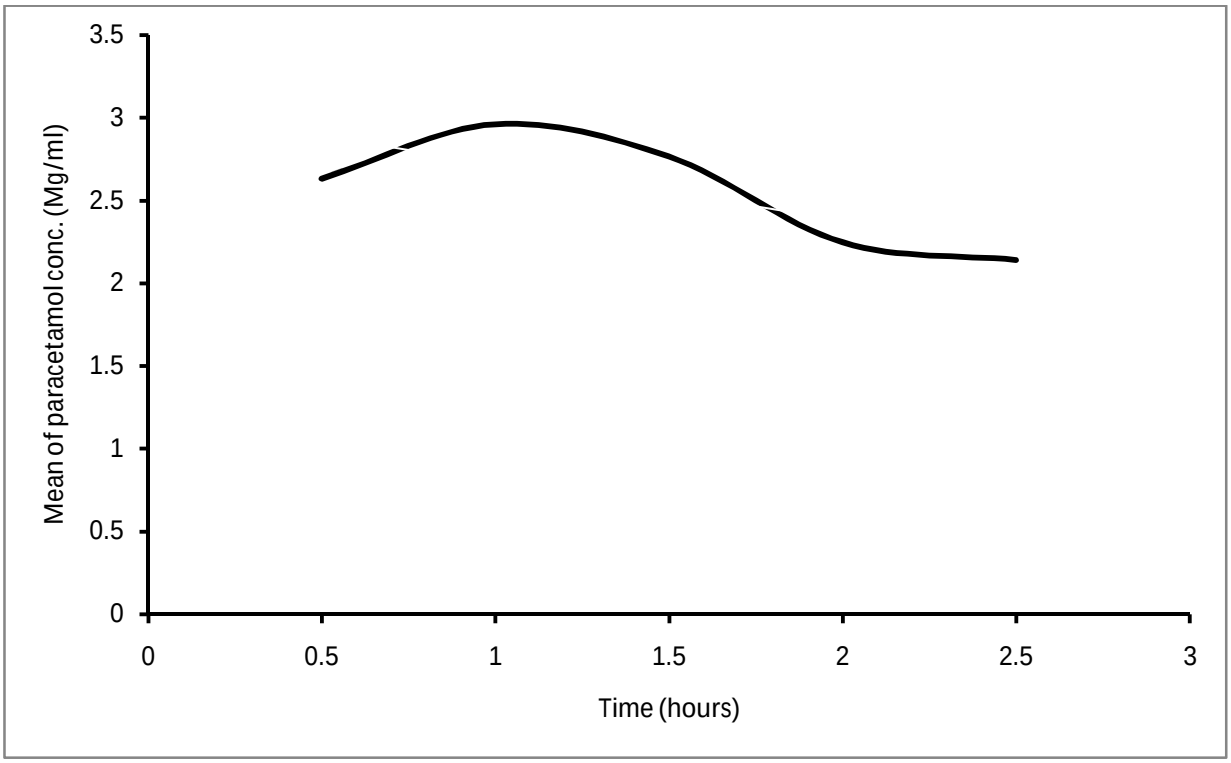


Figure 8: The curve of relationship between concentration of paracetamol and time at ½, 1, 1½, 2, 2½ hrs.

Table 2: Means, stander deviations and t-test for five groups which are taking paracetamol (1000 mg) plus vitamin C (240 mg)

Groups according to the sampling time	Means	Stander deviation	*t*-test	P value
Group (1) After half hours N=6	4.645	2.5093	4.534	<0.01 *
Group (2) After one hours N=6	4.3047	2.3167	4.552	<0.01 *
Group (3) After one and half hours N=6	2.7370	1.8868	3.563	<0.05 *
Group (4) After two hours N=6	2.4632	1.3688	4.408	<0.01 *
Group (5) After two and half hours N=4	1.6440	1.1445	2.873	0.064

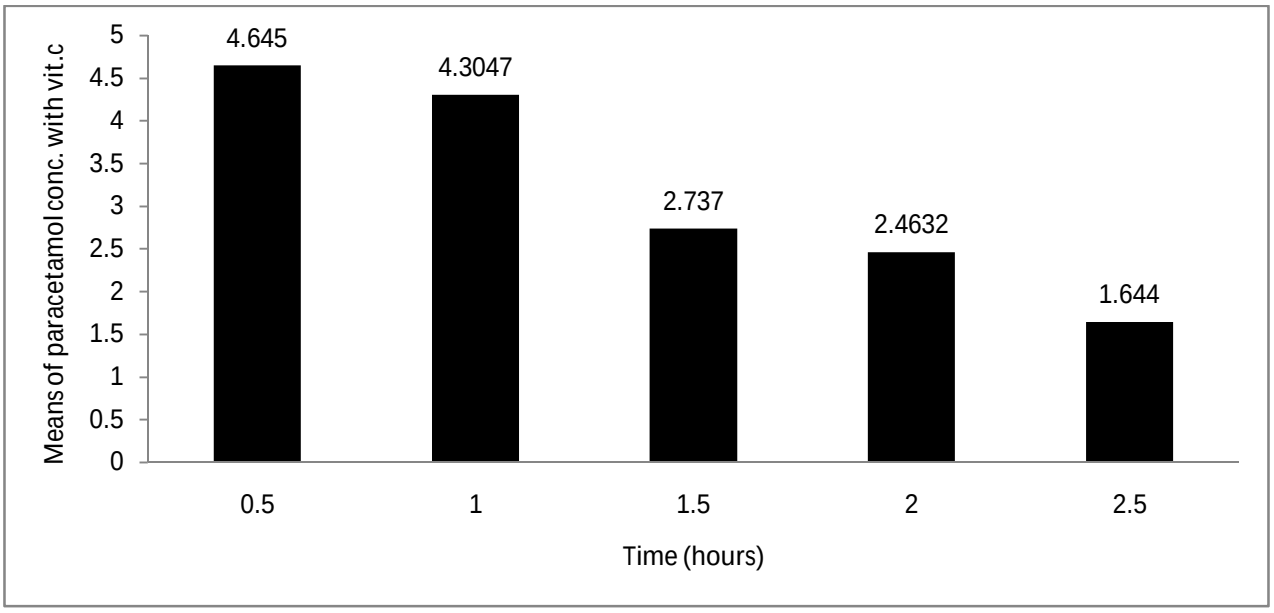


Figure 9: The relationship between the concentration of paracetamol plus vitamin C with time at ½, 1, 1½, 2, 2½ hrs.

Table 3: Means, stander deviations and t-test for five groups which are taking paracetamol (1000 mg) plus omega (1000 mg)

Groups according to the sampling time	Mean	Stander deviation	*t*-test	P value
Group (1) After half hours N=6	3.6445	2.5378	3.518	<0.05 *
Group (2) After one hours N=6	2.9153	1.8014	3.964	<0.05 *
Group (3) After one and half hours N=6	2.4950	1.5507	3.941	<0.05 *
Group (4) After two hours N=6	1.5973	0.905	4.323	<0.01 *
Group (5) After two and half hours N=6	2.6568	1.5983	4.072	<0.01 *

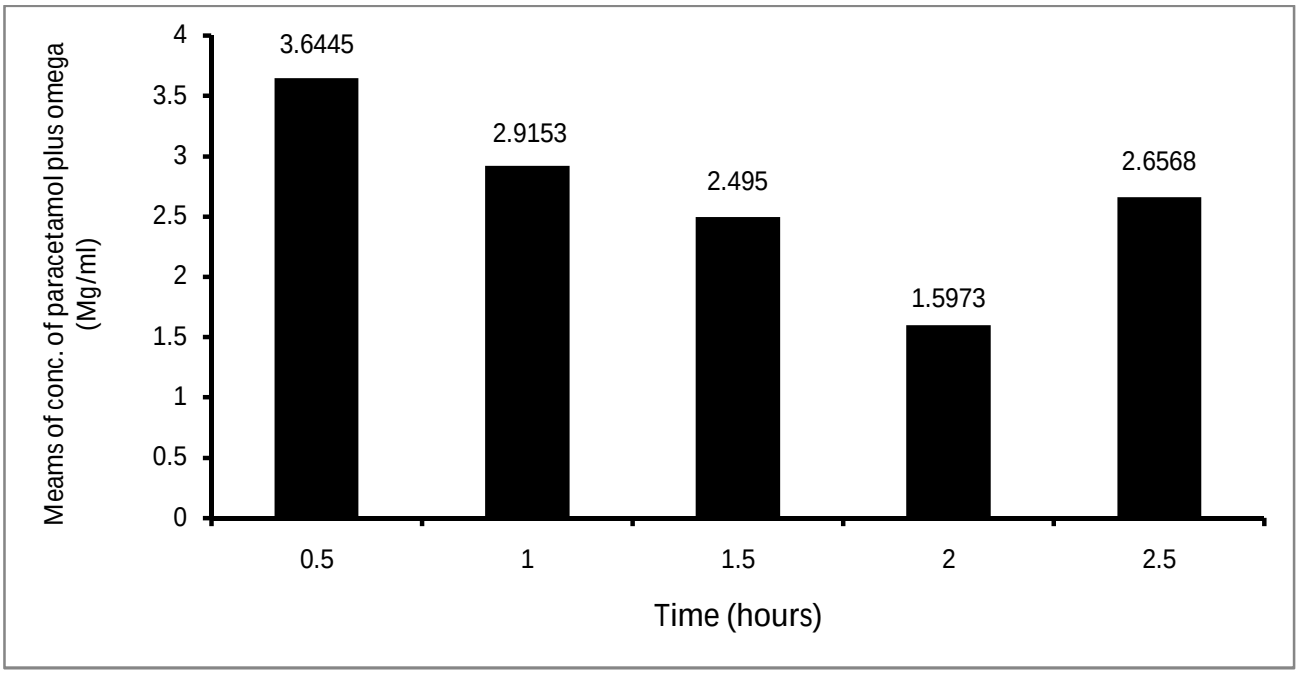


Figure 10: The relationship between the concentration of paracetamol plus omega with time at ½, 1, 1½, 2, 2½ hrs.

Table 4: Comparism between means and stander deviations for three groups (paracetamol (1000 mg), paracetamol (1000 mg) plus vitamin c (240 mg) and paracetamol (1000 mg) plus omega (1000 mg))

Time	Group of paracetamol only Mean ±St.dev	Group of paracetamol plus vit.c Mean ±St.dev	Group of paracetamol plus omega Mean ±St.dev	t-test	P value
After half hours	2.6352±2.5237	4.645±2.5093	3.6445±2.5378	2.558	0.051
After one hours	2.9638±2.0529	4.3047±2.3167	2.9153±1.8014	3.536	<0.05*
After one and half hours	2.7688±1.6201	2.7370±1.8868	2.4950±1.5507	4.186	<0.01*
After two hours	2.2508±0.8347	2.4632±1.3688	1.5973±0.905	6.605	<0.01*
After two and half hours	2.1433±1.0303	1.6440±1.1445	2.6568±1.5983	5.096	<0.01*

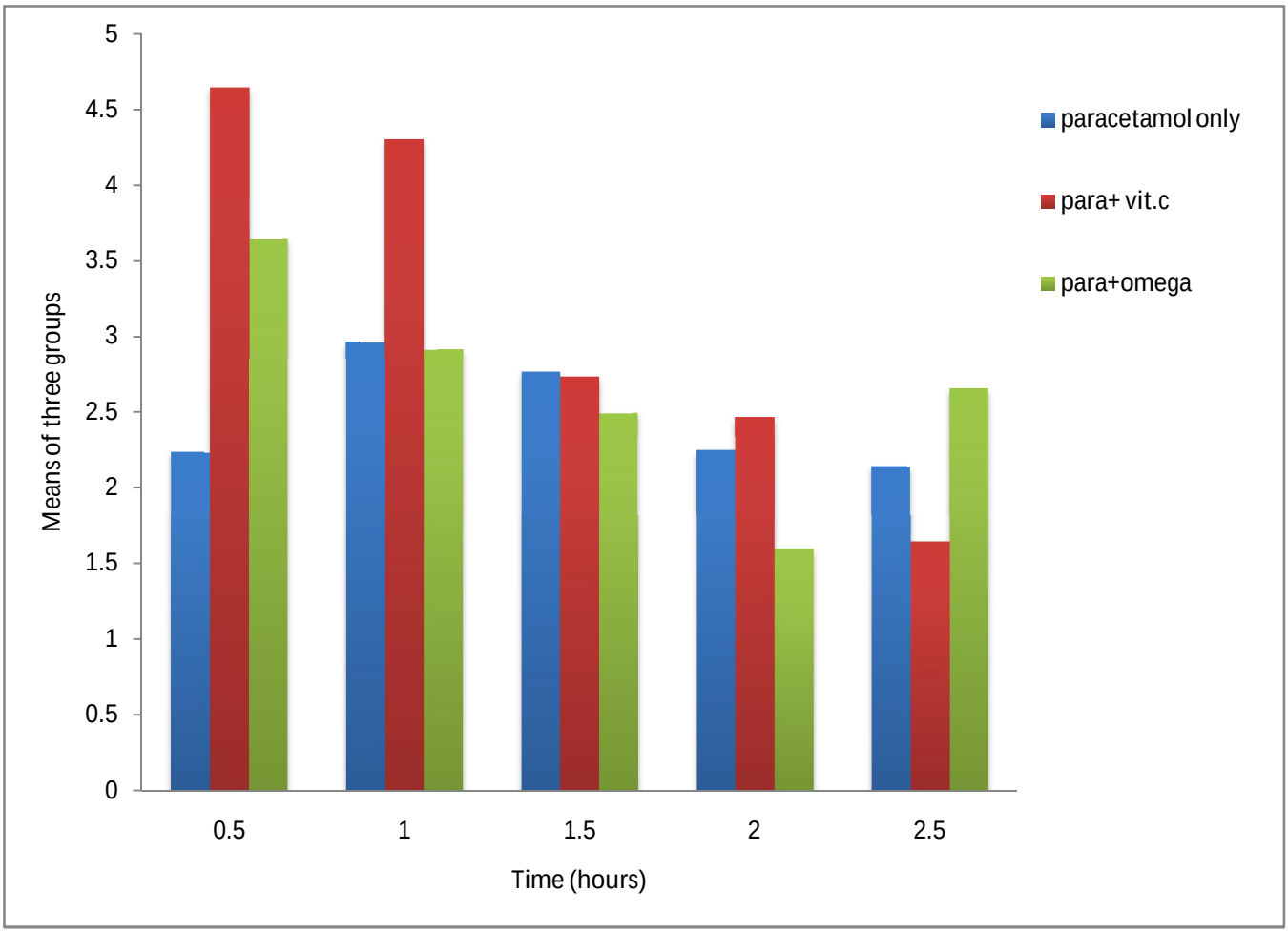


Figure 11: The relationship between the concentration of paracetamol, paracetamol plus vit C and paracetamol plus omega with time at ½, 1, 1½, 2, 2½ hrs.

4.Discussion:

Paracetamol is considered as one of the safest and most frequently used as analgesic and antipyretic drug all over the world. It is typically used for mild to moderate pain. However, many problems and concerns associated with its use appeared by time.[21]

The absorption of paracetamol is significantly increased with the time because subjects in this study were take the drug with empty stomach and they didn't have history of disaeses and drugs and the absorption of oral paracetamol from the gastrointestinal tract by passive transport and it is slowed if gastric emptying is delayed by food, posture, disease and drugs such as propantheline and narcotic analgesics,[22] therefore, the concentration of paracetamol were increased with time significantly.

The effect of vitamin c on the absorption and metabolism of paracetamol is significantly at different time, specifically at first half hour that mean vitamin c which is very strong antoxidant agent due to present enediol structure which can be oxidized easily to diketones.[23]

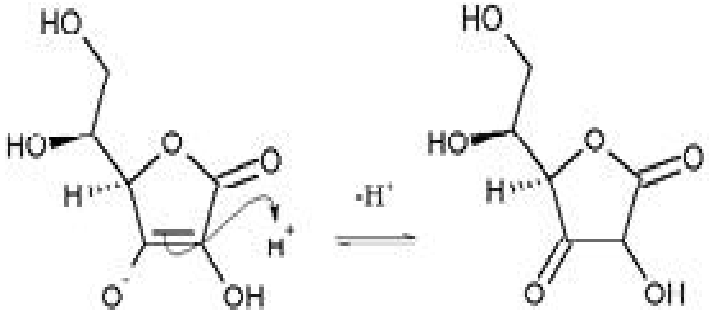


Figure 12: the mechanism of antioxidant activity of vitamin c

Antioxidant activity of vitamin c produce many mechanisms that affect on the metabolism of paracetamol like antoxidant has functions are concerned with the removal of free radical species such as hydrogen peroxide, superox-ide radicals, alkoxy radicals, and maintenance of membrane protein thiols and as a substrate for glutathione peroxidase (GPx) and GST, which is responsible for metabolism of

paracetamol.[24]Also antioxidant has reducing property of pro-oxidative enzyme of hepatocyte that increased of metabolism rate of paracetamol.[10 & 25]

The other antioxidant agent, omega 3 which is composed from 3 polyunsaturated fatty acids include eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA).[12] Also omega 3 affected on the kinetics of paracetamol which is appeared as the increasing of absorption and metabolism of it at different time significantly. Where unsaturated fatty acids possess potentially notable antioxidant activity.[26 & 27] Antioxidants are substances that have the ability to delay or inhibit oxidation processes, even when used in small amounts, reducing the reaction rate or extending its period of induction. The effectiveness of an antioxidant is directly linked to increasing or prolonging the induction period of oxidation reactions of a substrate and can be expressed as an antioxidant index or protection factor, In the presence of antioxidants, the oxidative rates decrease of glutathione due to an increased activation energy for reaction, thus increasing the "lifetime" of the substrate like paracetamol.[16] Also unsaturated fatty acid chain serves as a membrane stabilizer by preventing changes to the membrane fluidity as well as its conversion to lipid protective mediators in hepatocyte.[17 & 28]

When the comparism between the effects of vitamin c and omega 3 in the kinetics of paracetamol, we found that vitamin c had more potent effect than omega 3 at 0.5 and 1 hours. While the effect of vitamin c was became nearly to the effect of omega 3 at 1.5, 2 and 2.5 hours. This effect of vitamin c in the kinetics of paracetamol may be due to vitamin c is stronger antioxidant activity than omega 3.[15]

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