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PHARMACOKINETIC OF ACETAMINOPHEN IN RATS APPLYING A TWO-COMPARTMENT OPEN MODEL

Molina Trinidad, Eva María [1, 2*]; Ariza Ortega José Alberto[3], Vence González Patricia [3], Aguirre Renteria Saúl Alejandro[4], Balderas Delgadillo Carmen[5], Becerril Flores Marco Antonio [1], Almaguer Vargas Georgina[6], Montejano Rodríguez José Ramón [6].

[1]Academic Area of Medicine. Institute of Health Sciences. Autonomous University of the State of Hidalgo, Ex-Hacienda de la Concepción, Tilcuautla, Hidalgo, Mexico.
[2]Department of Engineering and Technology (Biopharmacy), Campus Cuautitlán, National Autonomous University of Mexico, Field 1, Av. May 1st. S / N, Santa María de Guadalupe, Las Torres Cuautitlán Izcalli, State of Mexico, Mexico.
[3]Academic Area of Nutrition. Institute of Health Sciences. Autonomous University of the State of Hidalgo. Ex-Hacienda de la Concepción, Tilcuautla, Hidalgo, Mexico.
[4]Faculty of Chemical Sciences. Durango Campus. Juárez University of the State of Durango. Av. Veterinaria S / N, University Circuit, Durango, Mexico.
[5]Academic Area of Dentistry. Institute of Health Sciences. Autonomous University of the State of Hidalgo. Ex-Hacienda de la Concepción, Tilcuautla, Hidalgo, Mexico.
[6]Academic Area of Pharmacy. Institute of Health Sciences. Autonomous University of the State of Hidalgo. Ex-Hacienda de la Concepción, Tilcuautla, Hidalgo, Mexico.

*eva_molina8849@uaeh.edu.mx

Abstract

Pharmacokinetic studies of a drug are important to assess the bioavailability in the body. In this work, the pharmacokinetics of acetaminophen were evaluated *in vivo*, in an animal model in *Wistar* rats induced with hepatocellular carcinoma (HCC) (n = 20) and healthy rats (n = 20) using a balanced design. Rats were administered approximately 250 mg/ kg/day (50 mg to 100 mg) and urine samples were obtained subjected to quantify p-aminophenol. The absorbance of each sample was obtained by spectrometric method and the pharmacokinetic parameters were determined. Values were fitted using a two-compartment model. Significant differences were found between the constants of healthy rats and those with hepatocellular carcinoma k_{10} (k_e) (0.0017 vs 0.0021 h^{-1}), k_{12} (0.033 vs 0.035 h^{-1}), k_{21} (0.011 vs 0.017 h^{-1}), k_a (0.053 vs 0.081 h^{-1}), t_α (2.3 vs 2.16 h), t_β (14.45 vs 17.02 h) and biodistribution volume Vd_1 (5.93 vs 8.64) and Vd_2 (19.23 vs 20.22). Light microscope images of liver tissues show affinity for liver-damaged rat tissues. There is the affinity of the drug with liver carcinoma where it had a prolonged elimination constant and an increased volume of distribution in rats with hepatocellular carcinoma. Verifying that it is effective and available and can serve as a biological biomarker in clinical diagnosis, since it reduces the pain generated by cancer. The pharmacokinetic data indicate that the open two-compartment model explains the behavior of the drug in healthy rats with hepatocellular carcinoma and may be useful in addressing the behavior of hepatocellular carcinoma.

Keywords: *acetaminophen, pharmacokinetic, hepatocarcinoma*.

Introduction

Acetaminophen is a metabolite of phenacetin and its analgesic and antipyretic action is used in patients with allergy or intolerant to aspirin (acetylsalicylic acid). Clinically has antipyretic and analgesic quick and effective in infants, children, adolescents and adults. This drug acts on the central nervous system. It´s believed to increase the pain threshold by inhibiting cyclooxygenase in peripherals tissues, lacks anti-inflammatory activity. Acetaminophen also appears to inhibit the synthesis and/or the effects of various chemical mediators that sensitize pain receptors to mechanical or chemical stimuli. Antipyretic effects of acetaminophen block endogenous pyrogen in the hypothalamic temperature control center, by inhibiting the synthesis of prostaglandins [1]. It does not cause gastric irritation, mucosal erosion and bleeding, which can occur after administration salicylates (salicylic acid). It does not affect the prothrombin time, viewed it has very peak action on platelets. It has been reported to have no effect on cardiovascular or respiratory system [2].

In this paper the behavior of drugs administered orally in *Wistar* rats is important to determine the amount of free drug that is available to act on a specific action site and result in a pharmacological effect on the body. For this reason, it is essential to know the reason of drug bioavailability in the body. The low bioavailability of a drug depends on the drug metabolism in the body, i.e., there are physiological factors that affect drug metabolism in the body, the site of absorption, the absorption rate refers to the pharmacokinetics of the drug, or the effect the first step (drug metabolism in the liver) and other factors. In this case, acetaminophen (N-acetyl-paraminophenol or N-(4-Hydroxyphenyl) acetamide) is one of the essential drugs according to the World Health Organization and in Mexico is used widely as an analgesic to control pain [3].

Regarding pharmacokinetics based on the literature it is reported that after oral administration, acetaminophen is rapidly and completely absorbed from the gastrointestinal tract [4]. Peak plasma concentration reached within 30-60 min, although not entirely relating to maximum analgesic effects. Acetaminophen is bound to plasma proteins in 25%, whereby 75% of the drug is in free or unchanged form.

Approximately one quarter of the dose of drug is metabolized in the liver (first pass effect). Also, this is metabolized in the liver the majority of the therapeutic dose, so glucuronide and sulfates conjugate, which are removed later in the urine. Between 10 and 15% of the dose undergoes oxidative metabolism by cytochrome P_{450} isozymes, then it is conjugated with cysteine and mercapturic acid; only 5% of consumed acetaminophen is oxidized in the liver by the P_{450} cytochrome leading to N-acetyl-p-benzoquinoneimine (NAPQI), which causes hepatoxicity [5]. After an overdose, depletion liver glucuronides and sulfates when there is malnutrition, or alcoholism occurs, so that acetaminophen undergoes oxidative metabolism which is the most toxic, through CYP2E1 and CYP1A2 enzyme system [6, 7]. Also, is presented when this metabolite acetaminophen is administered with other inducer drugs in the liver. In renal failure metabolites may accumulate but not the unchanged drug [8, 9]. The elimination half-life of acetaminophen is from 2 to 4 hours in patients with normal liver function. In patients with liver dysfunction it is increased the half-life time. This drug is used for the symptomatic treatment of mild to moderate pain intensity and in cases of fever. Not indicated in patients hypersensitive to the drug [10], in patients suffering from liver damage or for viral hepatitis and in patients with renal impairment or suffering from anemia. It is also a drug to be used in the different treatment [11] and dose should be adjusted by the presence of metabolites. Based Anwar El-Shahawy et al. [12] the acetaminophen is known for its use in clinical, an example is being used as analgesic in infants [13]. Much information has been reported regarding the use of acetaminophen and pharmacokinetics of acetaminophen, however as persistent use in the clinic is important to know the intraindividual and interindividual population response study. The response to this drug

depends of the biological variability of every subject.

In this paper, we describe the pharmacokinetic of acetaminophen in one dosage form (tablet) and calculate the pharmacokinetics parameters *in vivo* follow the bicompartmental mathematical model and with the model in rats.

Methods

Materials and reagents

The formulation was produced in a pharmaceutic laboratory, considering one as patent reference. We used formulations of acetaminophen (250 mg) in tablets. Also, other reagents were of analytical grade as sodium hydroxide and hydrochloric acid. The acetaminophen standard was obtained by *Bruluart* laboratories.

Pharmacokinetic in rats

Rats were given approximately of 50 to 100 mg (dose/Kg) of acetaminophen orally and through the use of metabolic cages urine samples were taken. The absorbance of all the urine samples taken at 0.25, 0.5, 1.2, 2.3 and 24 h, were used as the input data for the *WinNonlin* program to calculate the pharmacokinetic parameters for a bicompartmental model following a single oral dose. The following pharmacokinetic parameters were determined: area under the curve (AUC_0^{∞}), apparent volume of distribution ($Vd_1$and Vd_2), half-live ($t_{1/2\alpha}$ and $t_{1/2\beta}$), T_{max} and Cp_{max}.

Animal model

Pharmacokinetic studies in *Wistar* rats were carried out according to the rules and regulations of the official Mexican standard 062-200-1999 [14]. Male Wistar rats (200-400 g) maintained on standard PM 5001 feed (Purina) was used according to the rules and regulations of the Institute for Pharmacokinetic Studies [15]. The rats were kept in metabolic cages with wood-shaving bedding. Temperature was maintained at 22° C and humidity at 65%, with a 12 h light-dark cycle. Water and feed were provided *ad libitum* [16].

Tumor induction in rats and histopathological studies

The HCC spread in the peritoneal cavity of normal rats to obtain ascites from *Wistar* rats and identify AS-30D hepatoma cells. The rats developed ascites in 15 days. The propagation process was repeated several times containing enough developed cells. Then ascites samples were taken that were taken to a culture medium to obtain bacterial growth and histological studies were performed to confirm the presence of hepatocarcinoma cancer cells such as those of AS-30D hepatoma. These cells were taken to a centrifugation process of approximately 7 mL of ascites. Then 1 million cells were taken in 0.1 mL of phosphate buffer at pH 8 to be administered subcutaneously in the dorsal part of the rat. Tumor growth and cell propagation were monitored. After inoculation the tumor developed three months after implantation. Tumors obtained from 7 rats were measured. Tumors of 0.3 to 0.8 cm in diameter and 1.4 g were detected. The pathology indicates that tumors developed due to the presence of the cell line obtained from ascites, since AS-30D cells are characteristic cells of rat hepatocarcinoma.

Histopathological studies performed to determine the histological nature of the tumor was based on the identification of the tumor due to infections or other nature. The technique used to identify damaged tissue was based on the use of dyes to have tissues such as hematoxylin and eosin fixed and with Masson´s trichromic solution in formalin and embedded in paraffin, making histological cuts of 0.05 µm of fragments (3.0 g) of rat tumors (n=7).

Analytical method

Samples collected during the monitoring pharmacokinetic were read directly on a UV-Vis spectrophotometer at a wavelength of 610 nm using Quartz cells. The urine samples in rats were identify to one spectrophotometer UV-vis and used cell of quartz follow the colorimetric method the assay was based in the enzymatic mechanism of ligands in the union with acetaminophen for

enzymatic action of aril acyl amidase and as product p-aminophenol and acetate. The p-aminophenol react with the 8-hidroxiquinoline-5-sulfonic with manganese ions to produce the [5-(4-iminophenol)-8-quinolona]. This was an indirect reaction because the quantification to realize with [5-(4-iminophenol)-8-quinolona], where the compound was proportional to quantify the acetaminophen [17, 18]. To carry out the identification reaction the samples were centrifuged at 2000 rpm and the supernatant was removed and then reacted with the p-aminophenol with the 8-hidroxiquinoline-sulfonic.

Statistical analysis

The data obtained from the urine samples underwent statistical analysis to obtain average absorbances and concentrations obtained to determine the standard deviation and %CV. T-test was performed to test the linearity of the calibration curve with determination of slope (m) and the intercept (b); and the ANOVA test for study of repeatability in the simulations during the procedure, considering a statistical criterion probability of $p<0.05$. To determine the pharmacokinetic parameters were used a fitting with compartmental model mathematic in the *WinNonlin* program.

Results

Pharmacokinetic in rats

A compartmental model following a single oral dose with first-order elimination was used to estimate value the pharmacokinetic parameters acetaminophen in healthy rats using *WinNonlin* program: plasmatic maximum concentration, Cp_{max}, maximum time, T_{max}, elimination half-life, $t_{1/2(\beta)}$, distribution volume, V_{d1} and V_{d2} were significantly different between groups for studies *in vivo* with hepatocellular damage and healthy rats (see Figure 1).

Analytical method

The analytical method was based on the absorption properties of analyte used (acetaminophen), since the maximum absorption was measured at a wavelength of 610 nm, based in their physicochemical properties determinate for a spectrophotometric method with the colorimetric reaction with 8-hidroxiquinolina-5-sulfonic with manganese (Mn). The analytic method was through of measure spectrophotometric in the range of visible .

Histopathological observations

Histopathological studies confirmed the identification of rat induced hepatocarcinoma. In the ascites fluid samples taken to the slides atypical nests were observed in a high-resolution optical microscope with 100-fold magnification to identify and in a 100X objective to confirm the hyperchromatic and pleomorphic epithelial cancer cells with round nuclei and with different quantities of cytoplasm. No bacterial presence or blood cells were observed, nor mitotic phase cells. Cancer cells were seen in the peritoneum of the rat in which a decrease in mitochondria was observed than in healthy hepatocarcinoma-characteristic cells.

Statistically analysis

Statistical analysis was based on the evidence statistically analysis of variance to verify that the pharmacokinetic parameters calculated in the formulation were different with hepatocellular carcinoma and healthy rats with acetaminophen, based on a balance design. *WinNonlin* program was used to determine the pharmacokinetic parameters and determine the bioavailability of acetaminophen *in vivo*. We used the Kruskal Wallis test and the "t" test to analyze the differences between groups or populations with pharmacokinetic obtained.

T-test was performed to test the linearity of the calibration curve with determination of slope (m= 0.048) and the intercept (b=0.039) how it showing in the Figure 2; and the ANOVA test for study of repeatability in the simulations during the procedure, considering a statistical criterion probability of $p<0.05$ ($F_{table}=3.49 > F_{AUC(0-\infty)}=2.65$, $F_{t(1/2\beta)}=1.93$, $F_{ke}=1.55$, $F_{Tmax}=3.25$ and $F_{Cpmax}=1.29$). To determine the pharmacokinetic parameters were

used a fitting compartmental program *WinNonlin*, to evaluate significance difference between groups.

The Kruskal-Wallis test indicated that the probability of having a value of $H \geq 63.2$ is less than probability of having a value of 0.47 ($P < 0.05$) due to the presence of the tumor observed in rats that developed hepatocarcinoma.

In the Table 1, we shown the pharmacokinetic parameters acetaminophen obtained in this study applying a bicompartmental model in healthy rats and with hepatocarcinoma. In the Figure 3 we observe the differences in cancer damaged tissue and the normal liver tissue in rats with the histological samples.

Discussion

It has been reported that the bioavailability of acetaminophen is dose dependent and increases at high doses due to the first-pass effect. Dhaneshwar et al. [19] reported the following pharmacokinetic parameters ke = 0.11 h^{-1} ± 0.03, Cp_{max} = 6.17 ± 1.47 µg/mL, T_{max} = 1.06 ± 0.72 h, AUC_0^{∞} = 31.69 ± 8.12 µg*h/mL and $t_{1/2}$ = 7 ± 2.10 h for a reference product in one study of bioequivalence and bioavailability; and ke = 0.09 h^{-1} ± 0.003, Cp_{max} = 6.02 ± 1.56 µg/mL, T_{max} = 1.22 ± 0.80 h, AUC_0^{∞} = 31.36 ± 7.70 µg*h/mL and $t_{1/2}$ = 7.73 ± 1.64 h for the product under study. The following data reported from 1974 by Kenneth & Albert and et al. [20] is ke = 0.324 ± 0.146 h^{-1} following an open two-compartment model in plasma. Singla et al. [21] reported the following pharmacokinetic parameters Cp_{max} = 12.3 µg/mL, T_{max} = 1.0 h, AUC_0^{∞} = 44. 4 µg*h/mL, $t_{1/2}$ = 2.53 h and Cl = 24.6 L/h.

Previous reports consider the importance of *in vivo* studies pre-clinic phase to compare the different formulations currently used in medical practice at the time to select the appropriate drug knowing the characteristics of bioavailability and bioequivalence, since this drug is still used in clinical practice.

The values of elimination constant (k_e) reported in this paper are: k_{10} = 0.0017 ± 8X10^{-4} h^{-1} to healthy rats and with hepatocarcinoma k_{10} = 0.0021 ± 3X10^{-4} h^{-1} (See Table 1). The low k_{10} values in the different studies indicated that the concentration of acetaminophen reaches a maximum absorption and then slowly eliminated in a certain time whose speed indicates the process of elimination of the drug. With respect to the other pharmacokinetic parameters, such as the area under the curve, the following behavior was presented AUC_0^{∞}= 789 ± 108 µg*min/mL > AUC_0^{∞}= 636 ± 79 µg*min/mL, the calculation was obtained by applying the method of residuals for an oral dose, using the non-linear *WinNonlin* program. We could observe that in healthy rats the value of AUC_0^{∞} is greater than the hepatocarcinoma AUC_0^{∞} in rats, so this formulation is more bioavailable because $F_{healthy}$ = 91.02 > $F_{hepatocarcinoma}$ = 76.75. The maximum absorption was 1.20 h and 0.5 h for healthy rats and hepatocarcinoma, respectively.

The half-life ($t_{1/2\ \beta}$) in the formulation under study was: $t_{1/2\beta}$= 14.45 ± 10.63 h $t_{1/2\beta}$= 17.02 ± 1.74 h, these data indicate that the elimination rate is prolonged with the tumor, that this drug can be used as a biomarker in the diagnosis and detection of cancer, for example. The behavior of Cpmax was the following healthy Cp_{max} = 72.82 ± 5.62 µg/mL > $Cp_{max\ hepatocarcinoma}$ = 66.72 ± 4.07 µg/mL. From the previous results, we can say that the bioavailability of paracetamol is greater than greater than 1 (F = 1.24), therefore, paracetamol is bioavailable.

In this study we determinate the significance difference between pharmacokinetic parameters obtain used acetaminophen in healthy and with hepatocarcinoma rats. The significance level was of 0.05 to make the statistical analysis. To check if there is a significant difference between the pharmacokinetic parameters obtained was found no significant difference between the groups and there is no significant difference between the pharmacokinetic parameters ($F_{table} > F_{test}$) with the ANOVA test. Therefore, the use of acetaminophen can help to detection of damage liver tissue, since is fixed in the tumor, as seen in the images obtained by optical microscopy in liver tissues with hepatocarcinoma cells (see Figure 3).

Finally, in this work we determinate of pharmacokinetics parameters in order to publish the pharmacokinetics of acetaminophen in a preclinical study in an animal model to verify that since this drug produces analgesic and toxic effects in could be a way to detect cancer or other abnormality related to liver damage, since this drug it has great affinity for muscle and for the tumor under study. It has not been studied in patients with impaired liver function. Although the factors of theoretical risk of hepatotoxicity due to acetaminophen in patients with chronic liver disease include: decreased metabolism of acetaminophen, increased system activity cytochrome P_{450} enzymes or depletion of glutathione deposits and for which one of the indicators when conducting studies with acetaminophen is to monitor the liver function in patients with disease liver [22, 23]. However, pharmacokinetic studies are important because they are used extensively in febrile processes and in pain control in pediatric patients and in older adults. Its use in patients with autism, attention deficit and hyperactivity disorders are also associated [24, 25].

Regarding the pharmacokinetic parameters obtained indicated that there is a significant difference between pharmacokinetics parameters. The compartmental model is a model available for elaborating and determined pharmacokinetics parameters of acetaminophen *in vitro* and *in vivo*. The experimental study in rats shown in the animal model that differences in dates of pharmacokinetics parameters can serve for identify the differences in the biological variability and the half-life could be used to calculate the therapeutic dose following the dosing regimen in the appropriate therapeutic window. Then exist a difference between pharmacokinetics parameters obtained *in vivo* with healthy and hepatocellular carcinoma in rats. The importance of obtaining the pharmacokinetic parameters of acetaminophen is because it is widely used in the clinic for pain control in children and adults. In addition, studies in the preclinical stage allow us to scale the behavior of drugs to give continuity in the clinical phase considering the biological variability.

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Two compartment open model, *WinNonlin*. Study *in vivo* in rats						
Pharmacokinetics parameters	**Healthy**			**Hepatocarcinoma**		
	Estimated	SE	% CV	Estimated	SE	% CV
A (µg/mL)	48.88	9.19	14.25	335.36	14.29	4.26
B (µg/mL)	15.08	2.13	16.07	14.34	1.71	11.92
AUC_{all} (µg*min/mL)	789	108	15.17	636	79.89	12.55
T_{max} (h)	1.20	0.16	13.33	0.50	0.057	11.4
Cp_{max} (µg/mL)	72.82	5.62	7.72	66.72	4.07	6.10
k_{10} (h^{-1})	0.0017	$8X10^{-4}$	4.74	0.0021	0.0003	15.66
k_{12} (h^{-1})	0.0033	$5X10^{-4}$	15.22	0.035	0.0051	8.53
k_{21} (h^{-1})	0.0011	$3X10^{-5}$	2.74	0.017	0.0018	10.84
k_a (h^{-1})	0.053	0.0011	2.17	0.081	0.0019	2.36
t_α (h)	2.3	0.16	6.96	2.16	0.24	11.09
t_β (h)	14.45	1.54	10.63	17.02	1.74	10.22
Vd_1 (mL)	5.93	0.89	15.01	8.64	1.09	12.61
Vd_2 (mL)	19.23	1.36	7.07	20.22	1.89	9.35

Table 1. Pharmacokinetics parameters obtained of acetaminophen *in vivo* with *Wistar* rats two compartmental absorption model.

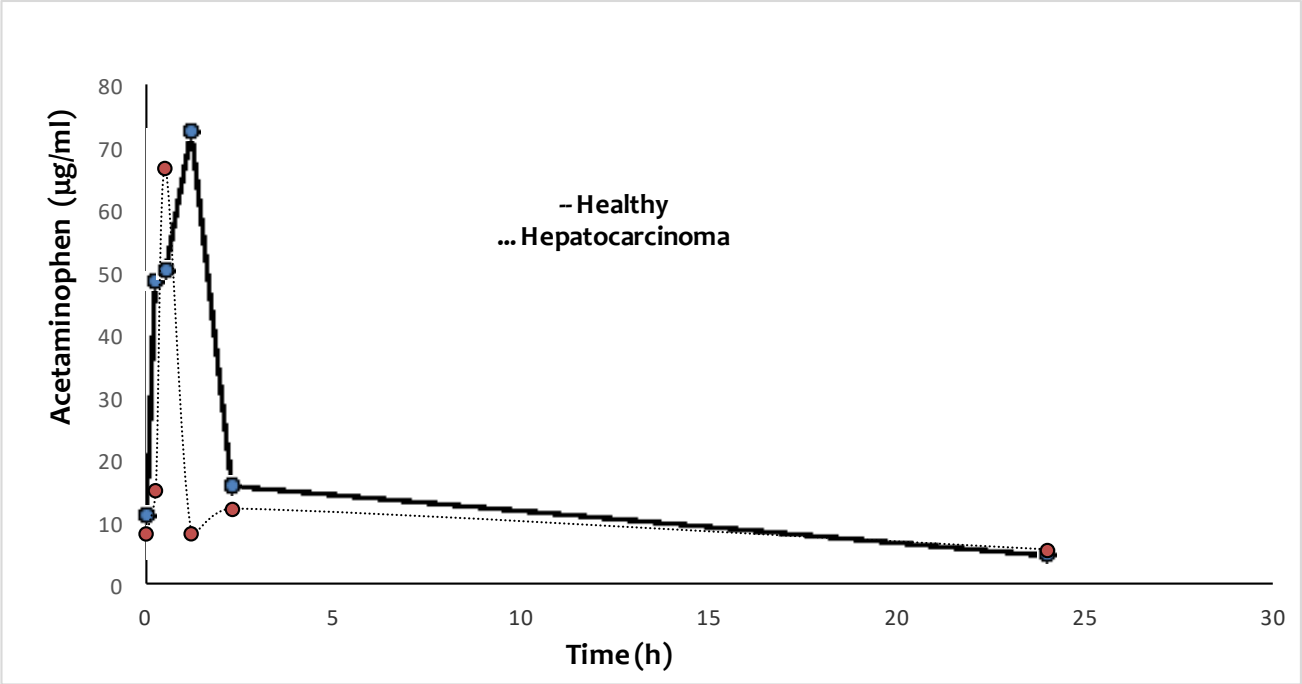

Figure 1. Pharmacokinetic profile healthy rats and in rats with hepatocarcinoma. Urinary concentration-time curve following oral administration of Acetaminophen (Dose: ≈ 50 at 100 mg/Kg/day). Profiles were obtained from experimental data with *WinNonlin* program.

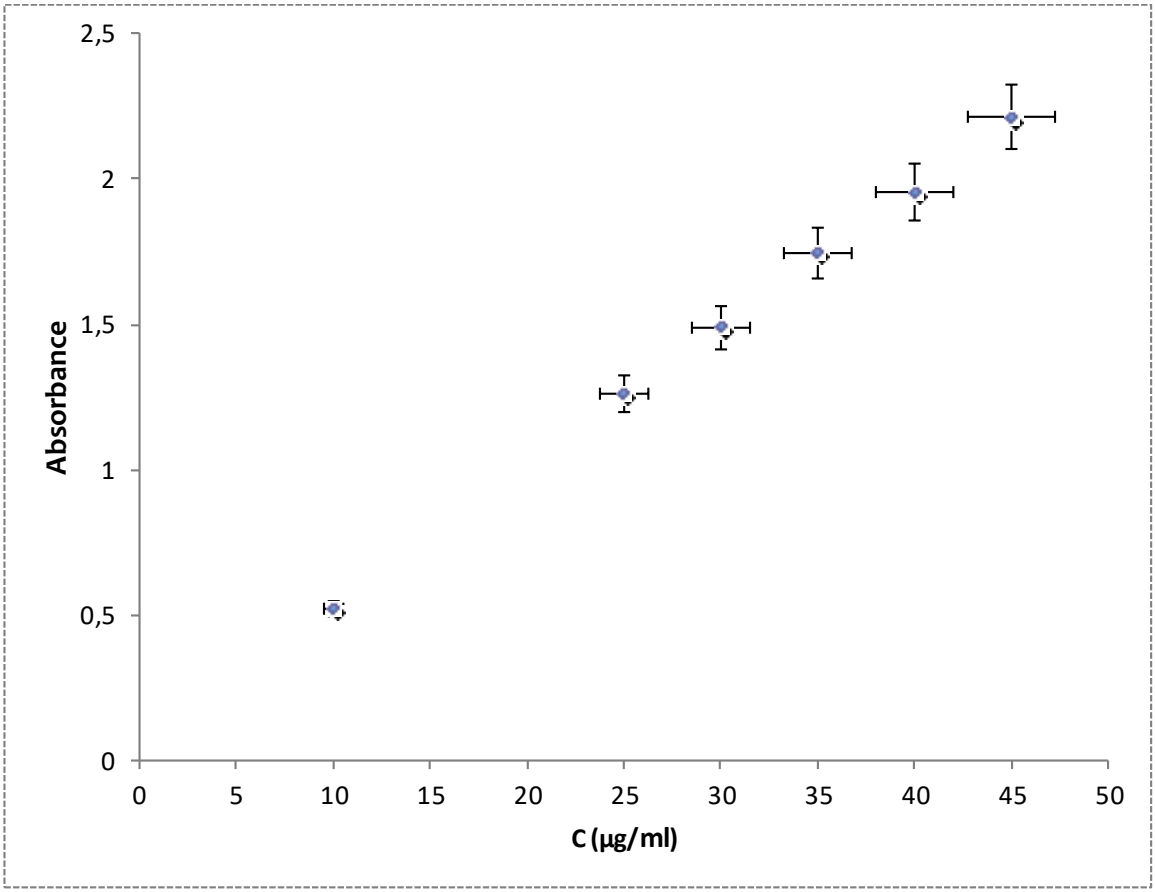

Figure 2. Calibration curve of acetaminophen by the proposed analytic method (R^2 = 0.99, %CV = 2.42).

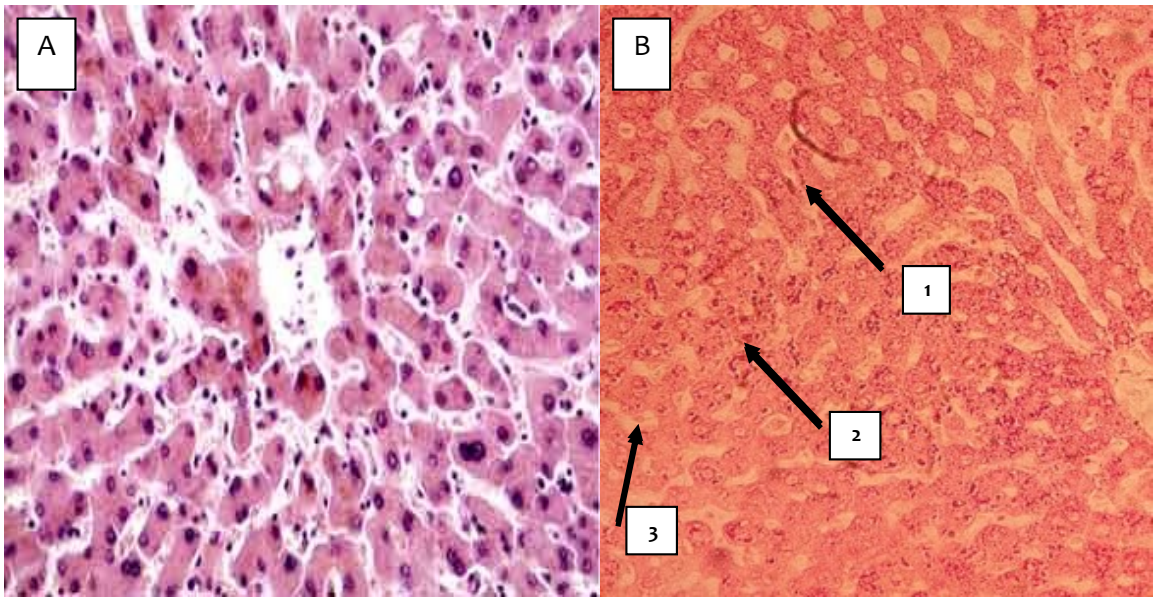

Figure 3. Normal liver tissue (A) and damaged tissue (B): (1) necrosis with absence of hepatocyte cytoplasmic membrane, (2) nuclear chromatin condensation, (3) yellowish brown pigment; stained with hematoxylin-eosin and Masson´s trichrome solution (100X) respectively.