Iraqi Journal of Physics, 2021 *Vol.19, No.48, PP. 107-114*

DOI: 10.30723/ijp.19.48.107-114

The Toxic Effect of Pb Nanoparticles Prepared by Laser ablation on Some Biochemical Aspects in Rats

Fiham Jassim AL-Obaidi[1], Abid A.Thaker[1], Asmiet Ramizy[2]

[1]Department of Biology, College of Science, University of Anbar

[2]Department of Physics, College of Science, University of Anbar

E-mail: sc.thakerscianb@uoanbar.edu.iq, asmat_hadithi@uoanbar.edu.iq

Corresponding author: fihamjasem86@gmail.com

Abstract

This work aimed to prepare and study the characteristic feature of lead nanoparticles (PbNPs) and follow its effects on some physiological aspects in rats. PbNPs was prepared by laser ablation of pure lead mass with a pulse of 500 and 100 mJ of energy. The results indicated that the wavelength was approximately 196 nm and the concentration was reported at 53.8967 mg / L. AFM, as the average diameter has been estimated at 69.93 nm. EFSEM shows the spherical shape of the particle. The experimental animals (rats) were divided into two groups, with seven rats for each one. The first group was a control and the second group was injected with 1 milliliter of PbNPs (53.8673 mg/l) per day for 45 days. Bioaccumulated lead (in liver, spleen kidney and muscles), total serum proteins, albumin, ALT, AST, ALP and antioxidants (GSH, SOD, CAT, and MDA) were estimated. By comparison to the control, PbNPs exposed animals. There were no significant differences in the bioaccumulated lead. In contrast, there was a significant reduction in total serum protein, albumin, GSH, SOD, CAT. Also, there was an increase in serum ALT, AST, ALP and MDA.

Key words

Lead nanoparticles, PbNPs, wistar rats, enzyme, protein, antioxidant.

Article info.

Received: Oct. 2020
Accepted: Jan. 2021
Published: Mar. 2021

التأثير السام لجزيئات الرصاص النانوية المحضرة بالاستئصال بالليزر على بعض الجوانب البيوكيميائية في الفئران

فهام جاسم العبيدي[1]، عبد علي ذاكر[1]، عصمت رمزي[2]

[1]قسم الأحياء، كلية العلوم، جامعة الأنبار

[2]قسم الفيزياء، كلية العلوم، جامعة الانبار

الخلاصة

يهدف هذا العمل إلى إعداد ودراسة الخصائص السامه لجسيمات الرصاص النانوية (PbNPs) ومتابعة آثارها على بعض الجوانب الفسيولوجية في الجرذان ، حيث تم تحضير PbNPs عن طريق الاستئصال بالليزر لكتلة الرصاص النقية بنبضة 500 و 100 مللي جول من الطاقة. ومن خلال النتائج تبين أن الطول الموجي للجسيمات المحضرة كان حوالي 196 nm وان التركيز كان 53.8967 mg/L ،في حين سجل AFM متوسط قطرحوالي 69.93nm واظهر EFSEM الشكل الكروي للجسيم. قسمت حيوانات التجربة (الجرذان) إلى مجموعتين، كل واحدة منها سبعة جرذان. كانت المجموعة الأولى عبارة عن مجموعة ضابطة والمجموعة الثانية تم حقنها بـ 1 ملليلتر من PbNPs يوميًا لمدة 45 يومًا. اذ تم تقدير الرصاص المتراكم حيويا في كل من (الكبد و الكلى والطحال والعضلات)، وتم تقدير البروتين الكلي، الألبومين، ALT، AST، ALP ومضادات الأكسدة

(GSH، SOD، CAT، MDA) وتبين عند مقارنة الحيوانات المعرضة لـ PbNPs مع حيوانات السيطرة لم تكن هناك فروق ذات دلالة إحصائية في الرصاص المتراكم حيويا. في المقابل، كان هناك انخفاض كبير في البروتين الكلي و الألبومين و GSH و SOD و CAT. في حين كانت هناك زيادة في مصل ALT و AST و ALP و MDA.

Introduction

Nowadays, nanotechnology, including nanoparticles, has increased widely in different fields. Exposure to lead can result in significant adverse health effects in organ systems [1]. Lead nanoparticles (PbNPs) are known as an important industrial material, which has been widely utilized in different human being activities [2, 3].

All of the prodact nanopartical activities are significant sources of the environmental pollution by lead nanoparticles besides that exhausted from the cars or Nanoscale lead (Pb) as a corrosion product formed inside lead-bearing pipes in drinking water distribution systems [4].

The released lead nanoparticles might reach the human being or animals through skin or drinking water or respiration or ingested food, and bind to different biological systems. When PbNPs becomes in contact with biological systems, their properties mainly changed, reflecting on their effects [5]. Lead nanoparticles usually have different assets than the bulk material and this due to its small size nature [6].

The previous studies found that the exposed animals to the lead (lead salts) nanoparticles accumulated significantly in organs and caused histological, biochemical and physiological changes [7, 8 and unpublished results].

Lead nanoparticles may be synthesized using three different methods: physical, chemical, and green (biological) methods. Their toxicity may be affected by the characters of the produced nanoparticles [9].

Almost all the previous studies used the salts of lead nanoparticles to study their toxicity [10, 11].

In the present study, the laser ablation of compressed lead metal was used to prepare lead nanoparticles, which may be considered the first toxicity study using pure metal.

The laser ablation in water produces the colloids of spherical nanoparticles of pure metals and metal oxides. The water temperature has an important effect on the shape of the nanoparticles produced [12].

In vitro and in vivo studies showed that nanoparticle-induced lung injury and pulmonary fibrosis due t to the reactive oxygen species (ROS) production [13]. The accumulation of ROS induces oxidative damage of macromolecules and lipid, protein, and DNA peroxidation. If the oxidation happens in a cell, it may alter gene expression for metabolism pathways [14, 15]. Oxidative stress may disrupt major metabolic routes within target organs such as the liver or kidneys [16].

The study aims to contribute to finding the toxic effect of pure lead nanoparticles.

Experimental work

Nanoparticle preparation by laser

After completing the preparation of compressed sample by By the Home Made press device, with a purity of 99.999 and the size of 30 nanometer (the target), the lead (Pb) it was bombed in a medium of double distillation water it size was (15 mL) with a height about (8 mm) above the target disk surface. A condenser Nd-YAG pulse laser with wavelength 1064 nm used at energy of (100 mJ) and the pulse were (500) pulse. The colloidal solution becomes slightly cloudy (like smoke), indicating the formation of colloidal metallic nanoparticles, taking into account the size of the

container in which the disk is placed so that the sample is away from the laser source about (12 cm). To examine the prepared nanoparticles, a UV examination was performed using a machine Shimadzu's dual-beam UIR-210A, concentration by SHIMADZU's AA-7000, AFM by SPM-AA3000 Advanced Angstrom Inc., FESEM by (FEI Verios 460L) Supra 50 VP.

Experimental animals

A adult white male Swiss Wistar Rats (*Rattus norvegicus*), of age ranging between 12-14 weeks and with an average weight of 250 grams, were used in this experiment supplied by College of Veterinary Medicine - University of Tikrit, Iraq. The animals were kept under standard laboratory conditions (24 ± 1 ° C; 12h light and 12h dark; fed freely).

Design of the experiments

Two groups of animals were used with seven rats in each group. Group A or the control group was given distilled water. Group B was injected with 53.8967 mg/L of body weight/day for 45 days [17].

Animal dissection

After the end of the exposure period, the rats were left untreated for two days; on day 3, they were anesthetized with chloroform [18]. Dissected, their liver, kidneys, spleen, and muscles were removed and digested for lead estimation. Blood was drawn from the animals by stabbing their hearts. The serum was separated by a centrifugation (at 3500 rpm) for 10 minutes and was kept in the freezer at -20 °C until conducting chemical tests.

Determination of lead concentrations in tissues

The accumulated lead in rats' organs was determined with a Flameless Atomic Absorption spectrophotometer (Analytik Jena-Germany), following the method described by Josthna et al., [19]. The samples (of 1 gm weight) were digested by 2.5 ml of HNO_3 +0.5 ml $HCLO_4$ in a conical flask (100 ml). The flasks were left for an hour at room temperature, then placed on Hotplate at 100 °C until the appearance of red fumes, then the temperature was raised to 200 °C, until the formation of white vapor. The remaining yellow color liquid was dissolved in nitric acid and used for lead detection after suitable dilution.

Biochemical tests

Alkaline phosphatase (ALP), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) activities, total protein and albumin concentrations were determined by using the Spanish origin Biosystem A15 automated device. GSH was measured according to Ellman (22). SOD and CAT activities were determined by using the Elab science Analysis Kit. MDA was measured according to the method described by Nur Alam et al., [20].

Statically analysis

The statistical analysis in this study included calculating the mean and the standard error. Which were done using the statistical program, Spss, the twenty-one edition. The complete random design was used, and the mean of the coefficients was compared using a modified Least Significant Difference test at a 0.05 significant level.

Results and discussion

Nano particles physical tests

Ultraviolet (UV) light spectrum

The absorbance calculated by the (UV-Vis) device as a function of the laser pulse was used in preparing nanoparticles with the laser power constant of 100 mJ, as it is shown in Fig.1 that the 500 pulse records a 196 wavelength.

Concentration

Through examining results concentration of nanoparticles using the AA-7000 device as a function of the laser pulses used in the current study with the stability of the laser power 100, as the pulse concentration was (500 plus) (53.8967 mg / litre).

Atomic Force Microscope (AFM)

The morphological characteristics studied using the SPM-AA3000 (AFM) microscope of the used pulse, as Fig.2 showed a two-dimensional, and three-dimensional image of the prepared particles, It was found that the average size (average diameter) of the nanoparticles prepared in the liquid phase was 69.93 nm.

The Field Emission Scanning Electron Microscope (FESEM)

Examination results which made with a FESEM Supra 50 VP pulse microscope used in the preparation of lead (Pb) nanoparticles as shown in the pictures (3) for pulse 500 showed that the prepared particles are nanoparticles and this is consistent with the AFM examination. The results showed that the prepared nanoparticles were spherical in shape.

Energy-Dispersive X-ray spectrometers (EDX)

The results of the EDX examination in Fig.4 showed the presence of the Pb particle in its nanoscale form, The EDX diagram of Fig.4 for the nanoparticles showed the presence of lead nanoparticles and their high purity.

The absorbance, as a function of the number of laser pulses used, was high, and this indicates that the concentration was high at the pulse of 500, which is consistent with the concentration measurement. The concentration of the 500 pulse was (53.8967 mg/L) since the concentrations of PbNPs recorded an acceptable value. AFM recorded 83.61 nm showed that the shape of the nanoparticles was spherical, as the laser skimming efficiency at a pulse of 500 leads to the formation of small particles.

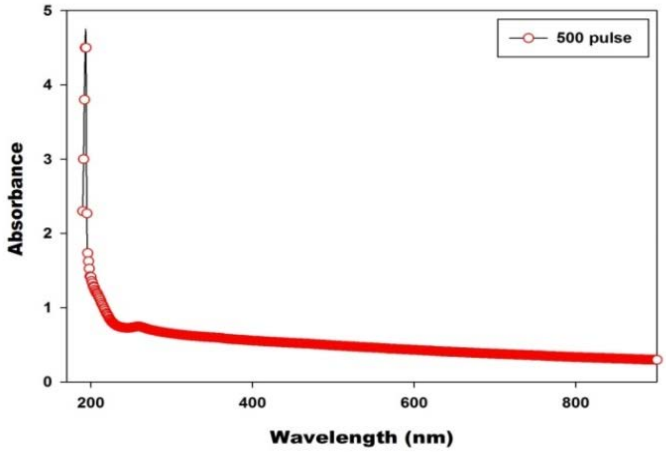
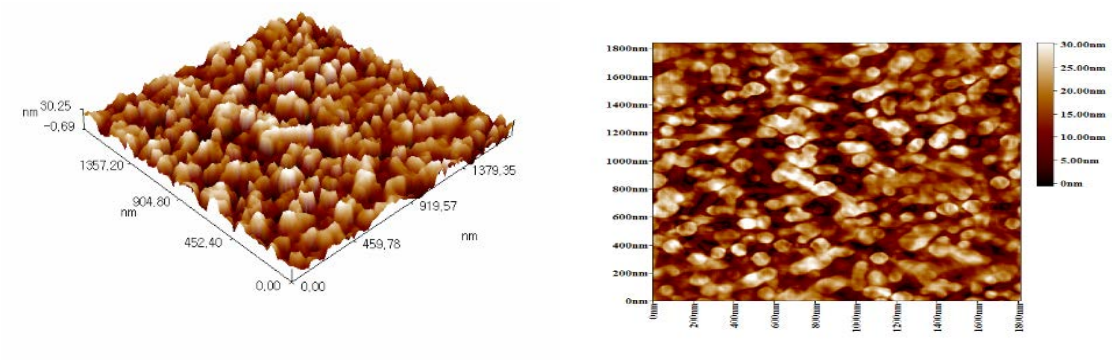


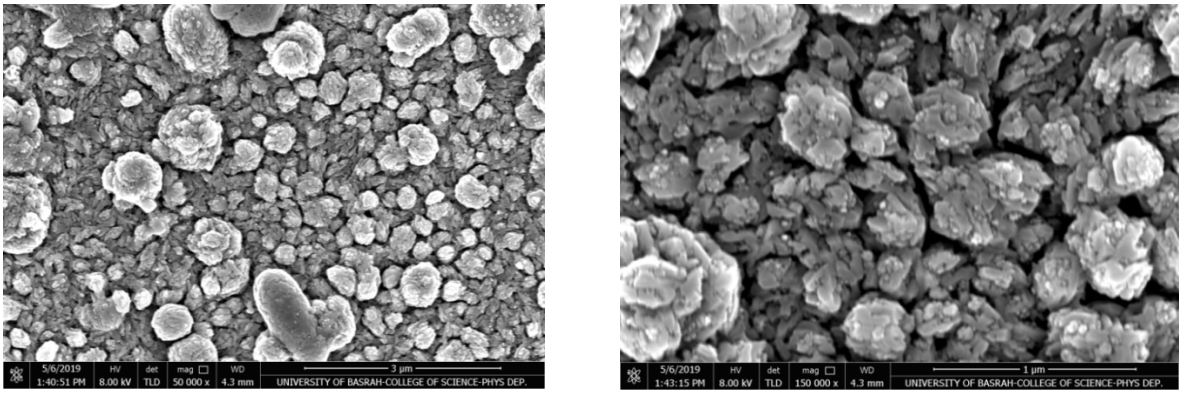
Fig.1: The absorbance of a laser pulse (500 pulses) (Energy (100 mJ).



(a) (b)

Amplitude parameters:
Sa(Roughness Average) 6.18 [nm]
Sq(Root Mean Square) 737 [nm]
Avg. Diameter: 69.93 nm

Fig.2: (a, b) 2D and 3D images of the prepared nanoparticles of the laser pulse (500 pulse).



(a) (b)

Fig.3: (a, b) Topographic composition and average size of the prepared laser pulse nanoparticles (500 pulse).

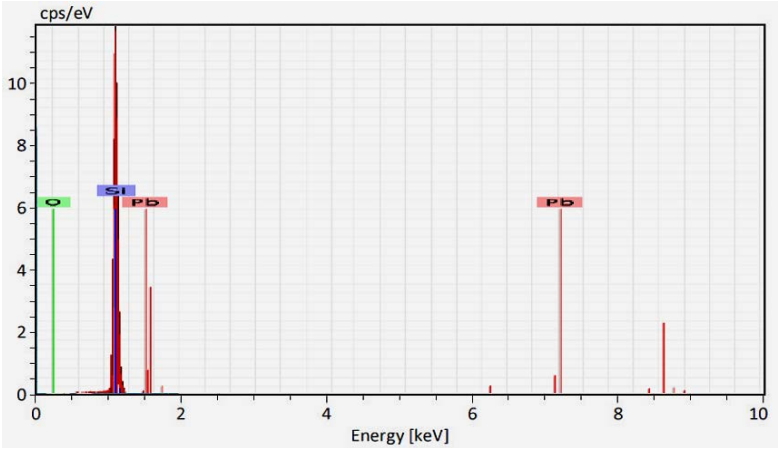


Fig.4: EDX spectrum analysis of lead nanoparticles.

Effect of PbNPs on proteins and enzymes

The current study results showed in Table 1 that there were no significant differences ($P < 0.05$) in the bio accumulated lead in different organs of rats as compared with the control group.

Table 1: Lead bioaccumulation in the organs of rats. In control and exposed animals to PbNPs.

Parameters Groups	Muscles (mg/kg)	Liver (mg/kg)	Kidney (mg/kg)	Spleen (mg/kg)
Control	6.4960±0.55923[a]	8.4043±0.46570[b]	6.8893±0.49005[c]	6.3002±0.52075[d]
PbNPs	7.6317±1.0117[a]	8.8193±0.63649[b]	7.5196±0.17725[c]	7.0715±0.20348[d]

Data are represented as mean ±SE n=7, P<0.05. Different letters refer to significant differences among the treatments

In spite of the no significant accumulation of lead, PbNPs caused a significant decrease ($P < 0.05$) in the concentrations of total serum proteins and albumin, and increase in the serum ALT, AST and ALP by compared to the control group (Table 2).

Table 2: Effect of PbNPs on the enzymes and proteins.

parameters Groups	AST (U/L)	ALT (U/L)	ALP (K.A.U./100ml)	Total protein (g/dl)	Albumin (g/dl)
A(Control)	47.2857±1.44279[a]	50.7143± 1.42619[a]	563.0000±35.6517[a b]	52.4286±2.3182 2[c]	36.9286±2.65570[c]
C (L.NP)	58.5714±1.86263[c]	55.6429± .50561[db]	679.5000±18.0755 c	41.5000±1.1073 3[b]	25.1429±1.03345[ac]

Data are represented as mean ±SE n=7, P<0.05. Different letters refer to significant differences among the treatments.

Table 3: Effects of PbNPs on antioxidants.

Parameters Groups	MDA µmol/L	SOD µmol/L	GSH µmol/L	CAT µmol/L
Control	2.3583±0.16137[a]	20.4239±1.19882[c]	208.8857±23.1697[b]	7.0765±0.33562[c d]
PbNPs	3.7458±.04812[b]	14.5124±1.43538[ab]	89.8143±12.2000[a]	6.2417±04390[ab]

Data are represented as mean ±SE n=7, P<0.05. Different letters refer to significant differences among the treatments.

The levels of the antioxidants GSH, SOD, CAT and MDA were estimated (Table 3). Comparing control group with PbNPs group, showed that the PbNPs group exhibited significantly elevated levels of hepatic MDA ($P < 0.05$).

When nanoparticles enter the body, they may combine, or interact with different cellular organelles or with the metabolic pathways affecting biological functions [12]. In this study, the exposure of rats to PbNPs revealed a significant decrease in the concentration of total protein and albumin. Changes in total protein values may indicate impaired liver function, with the liver being the main site for the synthesis of plasma proteins, mainly albumin [21]. Biomy et al., [22] mentioned that the decrease in protein level may be due to the liver's cirrhosis, leading to a reduction in the ability to synthesize protein.

The significant increase in the enzyme ALP above typical values upon exposure to PbNPs, this may be attributed to the body's inability to excrete it through the bile duct due to its blockage after the hepatocyte damage [23].

The increase of ALT and AST activities indicates damage to the tissues containing these enzymes, especially liver cells [24]. It was found that the stress caused by lead (or other toxicants) caused the production of free radicals, which interfere with the functions of the cells and increase cell membrane permeability [25, 26].

The decrease in the concentration of GSH might be due to the combination of lead with thiol groups (SH-) that protect cells against external damage after participating in the formation of GSH, which is an important antioxidant [26]. The decrease in the CAT enzyme may be due to lead inhibition of the active group (SH), which is essential for its work [27].

The decrease in the concentration of SOD may be attributed to the interference of lead with minerals which are essential for the enzyme action [28]. The increase in MDA concentration may be attributed to an increase in the production of free radicals, which leads to an increase in lipid peroxidation and then increased oxidative damage induced in cellular membranes leads to an increase in MDA concentration [29].

Acknowledgments

The authors would like to express their thanks and gratitude to the laboratories of the Department of Physics, College of Science, University of Anbar.

References

[1] M. Lippmann, "Environmental Toxicants: Human Exposures and their Health Effects". Hoboken: Wile, (2000).

[2] M. Sonmez, R. Kumar, Hydrometallurgy, 95 (2009) 53-60.

[3] B. Sljukić, CE. Banks, A. Crossley, RG. Compton, Analytica Chimica Acta., 587 (2007) 240-246.

[4] J. Senvaitiene, J. Smirnova, A. Beganskiene, A. Kareiva, Acta Chimica Slovenica, 54 (2007) 185-193.

[5] B. Jaffe, R. Roth, S. Marzullo, Journal of Research of the National Bureau of Standards, 55, 5 (1955) 239-254.

[6] WH. Dumbaugh, JC. Lapp, Journal of the American Ceramic Society, 75, 9 (1992) 2315-2326.

[7] R. Tayebee, B. Maleki, M. Sabeti, Journal of the Iranian Chemical Society, 14, 6 (2017) 1179-1188.

[8] D. Q. Ng, Y. Chu, S. W. Tan, S. L. Wang, Y. P. Lin, C. H. Chu, P. J. Chen, Environmental Science: Nano, 6, 2 (2019) 580-591.

[9] A. Asghar, S. Marzieh, Autumn, 2 (2015) 108-111.

[10] WF. Elbossaty, Journal of Medical Physics And Applied Sciences, 2, 2: 4 (2017) 1-4.

[11] J. Dumková, T. Smutná, L. Vrlíková, P. Le Coustumer, Z. Večeřa, B. Dočekal, M. Buchtová, Particle and Fibre Toxicology, 14, 1 (2017) 1-19.

[12] A. Amiri, M. Marziyeh, S. Marziyeh, Electronic Journal of Biology, 12, 2 (2016) 110-114

[13] Amra Bratovcic, "Synthesis, Characterization, Applications, and Toxicity of Lead Oxide Nanoparticles", In book: Lead Chemistry Publisher: InTechOpen, (2020).

[14] VY. Shur, EV. Gunina, AA. Esin, EV. Shishkina, DK. Kuznetsov, EA. Linker, Applied Surface Science, 483 (2019) 835-839.

[15] J. D. Byrne and J. A. Baugh, McGill Journal of Medicine, 11, 1 (2008) 43-50.

[16] M. Valko, D. Leibfritz, J. Moncol, M. T. D. Cronin, M. Mazur, J. Telser, International Journal of Biochemistry & Cell Biology, 39, 1 (2007) 4484.
[17] G. Ermak and K. J. A. Davies, Molecular Immunology, 38, 10 (2002) 713-721.
[18] D. Pelclova, V. Zdimal, Z. Fenclova, Occupational and Environmental Medicine, 73, 2 (2016) 110-118.
[19] G. Oszlánczi, A. Papp, A. Szabó, Inhalation Toxicology. Mar, 23, 4 (2011) 173-181.
[20] A. McKenzie, "History of Anaesthesia Society", The Royal College of Surgeons of Edinburgh. 3rd, (2011).
[21] P. Josthna, T. Geetharathan, P. Sujatha, G. Deepika, International Journal of Pharmacy & Life Sciences, 3, 12 (2012) 2186-2189.
[22] G. Ellman, Arch. Biochemistry and Biophyisics, 82 (1959) 70-77.
[23] M. D. Nur Alam, N. J. Bristi, M. D. Rafiquzzaman, Saudi Pharmaceutical Journal, 21, 2 (2013) 143-152.
[24] Milena Andjelkovic, Aleksandra Buha Djordjevic, Evica Antonijevic, Biljana Antonijevic, Momcilo Stanic, Jelena Kotur-Stevuljevic, Vesna Spasojevic-Kalimanovska, Milos Jovanovic, Novica Boricic, David Wallace, Zorica Bulat, Int. J. Environ. Res. Public Health, 16, 274 (2019) 1-21.
[25] E.S. Biomy, M.G. El-Sayed, A.A. El-Komy, International Journal of Basic & Clinical Pharmacology, 7, 12 (2018) 1-12.
[26] J.J. Saukkonen, D.L.Cohn, R.M. Jasmer, S. Schenker, J.A. Jereb, C.M. Nolan, C.A. Ploquin, F.M. Gordin, D. Nunes, D.B. Strader, J. Bernardo, R. Venkataramanan, R. Timothy, Am J Respir Crit Care Med, 174 (2006) 935-952.
[27] W. L. Arneson, J. M. Brickell, (2007), Assessment of Liver Function. In: Clinical Chemistry: A Laboratory Perspective, Arneson, W.L. and J.M. Brickell (Eds.), F.A. Davis Davis Company, Philadelphia, ISBN-10 : 233-66.
[28] S. Choudhury, S. K. Panda, Current science, 87 (2004) 342-348.
[29] I. Shimizu, M. Kamochi, H. Yoshikawa, Y. Nakayama, (2012) Gender difference in alcoholic liverdisease. In: Shimizu I (ed) Trends in alcoholic liver disease research: Clinical and scientific aspects. InTech-Open Access Publisher, Rijeka, Croatia, 23-40.