ORIGINAL ARTICLE

Effect of Zinc Oxide Nanoparticles on Liver Cirrhosis Induced by Carbon Tetrachloride in Albino Rats

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ABSTRACT

Aim: To evaluate anticancer potential of zinc oxide nanoparticles individually and in combination with various conventional antibiotics against different multidrug-resistant bacterial isolates and validation of clinical parameters for measuring treatment efficacy in rat organs (liver) through biopsy.

Study design: Experimental study

Place and duration of study: Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore from 1st July 2019 to 30th June 2020.

Methodology: Zinc oxide nanoparticles with the purity of 99.5% obtained from IMBB, used for preparing Stock suspension (1 mg mL^{-1}) freshly in phosphate-buffered saline (PBS, pH 7.4) and sonicated prior to exposure. Male rats purchased from PGMI, Lahore were housed in animal cages at animal laboratory in ventilated room maintained at 25 °C±2°C and 70%±10% relative humidity with 12-hour light/dark cycle.

Results: No tumor in the liver was found and best results were obtained in liver function test. Liver disorder which cannot be reversed known as Liver cirrhosis was also observed. This disorder was observed with regular scarring of tissues of liver along with nonstop regeneration. Some of the major factors like alcohol and viral infections, etc. are among major reason for Liver cirrhosis.

Conclusion: Zinc oxide nanoparticles are the nanoparticles which have ability to induce apoptosis as well as release of reactive oxygen species (ROS) in cells. Hence zinc oxide nanoparticles are considered as a great nanoparticle against cancer and microbes.

Keywords: Cancer, Zinc oxide nanoparticles (ZnO-NPs), Chemotherapy, Radiotherapy, Albino rats

INTRODUCTION

Cancer refers to cluster of disease invoking any part of human body. Microbe is not responsible for cancer; it is the defect in our DNA that can be insertion or addition or deletion inside. Furthermore, the tumor is categorized into two classes: Benign tumor that remains at the same part and malignant tumor that can spread to infect the whole body. Myeloma, lymphomas and leukemias are other types where no tumor is formed[1-3]. Liver cancer is the most important and leading cause of death worldwide. Patients die within a year therefore fatality rate caused by this disease is very high[4]. The large organ: liver, helps in metabolism and filtration of blood from digestive tract. Enzymes/Bile juice released through liver helps in digestion and metabolism. Production of protein for body to perform proper function is also released from liver.[5] Scarring of tissue occurs when the liver is being damaged and causes cirrhosis which can lead to death. Scarring results in abnormal functions of liver. Factors causing liver damage can be chemical and biological[6]. It is known that liver filters anything that goes to our digestive tract and alcohol abuse is one of major cause of liver cirrhosis due to itstoxic properties. Another established causative ofliver cirrhosis is a virus[7].

Nanoparticles and its applications have made a great progress against many diseases and problems linked with them. Biologically synthesized nanoparticles are found useful for many biomedical application.[8] These nanoparticles fight against bacterial disease and cancer. Most sunscreen creams also contain nanoparticles which help skin against UV light from the sun[9,10].

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Conventional treatments for cancer include chemotherapy and radiation each having own benefits, however it has different side effects such as loss of hair and many others.[10] Chemotherapy is synthetic chemical formulation used against the uncontrolled division of cell. Although it's effective on cancer cells yet has drawback to its use, as it also affects normal cells, germ cells and hair cells. On other hand radiotherapy is a process where radio waves are used for killing of cancer cells, specified at point of origin however normal cells around are also affected causing radical changes and other problems. Only treatment that does not affects the normal cells around it is the target of ZnNPs towards cancer cell, however there is a chance of causing toxicity if proper dose of nanoparticles is not maintained[11].

With many advantages like lifesaving competence and disease killing properties, nanoparticles may have disadvantageof spreading toxicity which can be dangerous. Nanoparticles with different sizes serve different purposes and their property changes such as small particles may be more toxic than the large sized. A nanoparticle with the size 25nm is said to be more toxic than particles with size of 100nm. For ZnONPs with 25nm in size have different effects on different stages of cell cycle like it decrease G1 phase but increases S and G2 phase of cell cycle. Increased activity of nanoparticles is associated with smaller size than the larger one.[12] Because of its toxicity it can be dangerous for some important organs in body. Absorption of ZnONPs occurs in kidney, liver and spleen. Large amount of such particles having a toxic property can also induce liver cirrhosis[13].

Biologically synthesized nanoparticles can be used in many fields and application can be observed in almost everything including tissue engineering. They are helpful in

gene delivery andone of important application as cancer therapy. Nanoparticles are used for drug delivery, biosensors, as antimicrobial agents and also for imaging technology. This research is focused on investigating ZnONPs and CCL_4 in liver cirrhosis. This will help in finding other methods for protection of liver using nanoparticles instead of conventional methods of chemotherapy and surgeries.

MATERIALS AND METHODS

The reagents zinc-oxide nanoparticles, carbon tetrachloride, phosphate-buffered saline (Pbs), formalin and olive oil are used. ZnONPs biologically synthesized particles were present in a powdered form. In vitro study has already been conducted by usage of these particles, and successful results were obtained. ZnONPs were weighed on the measuring scale accurately and it was suspended in 1.5ml micro centrifuge tube containing 1ml of PBS solution. ZnONPs were placed for sonication of around two hours on the sonication machine. 1.5ml micro centrifuge tube was suspended in the water bowl, and tip of sonication machine was dipped inside. When sonication process was competed, process of vortex was performed. Nanoparticles were vortex for twenty minutes for the first time. Each day of dosage nanoparticles were placed in the sonication machine for one hour and vortex for ten minutes.

Preparation of Carbon Tetrachloride (CCl_4): Carbon tetrachloride was taken in a 50ml falcon. One ratio one concentration of carbon tetrachloride was prepared with pure 100% pure olive oil. CCl_4 is a toxic substance, and one shouldnot use it without gloves. It can cause serious irritation if it comes in contact with skin.

Animal model was common laboratory rat which was of pure breed and albino (biological name Rattusnorvergicus). Rats takenwere of almost similar weight and divided into three groups: NORMAL, Carbon tetrachloride (DISEASED), ZnONPs and CCl_4 (Carbon tetrachloride)combination. After 14 days, all rats were euthanized. Liver organ and blood of each rat was obtained. It was sent for histopathology slides and blood tests respectively.

Blood taken from the heart by inoculating 10cc syringe directly into heart was poured into collection tubes and sent for blood testing. Liver of rat was taken out and some portion of it was fixated into formalin for histopathology.

ASAT (GOT) FS*is an optimized UV test according to International Federation of clinical and laboratory medicine.

Alanine Aminotransferases (ALT).Kinetic test was performed according to International Federation of clinical and laboratory medicine.

Total bilirubin: Performed by sung 2, 4 dichloroaniline (DCA). Alkaline phosphatase conducted photometric test optimized standard method according to the German Society of Clinical Chemistry (DGKC). The data was entered and analyzed through SPSS-22.

RESULTS

Weight of Rats: Body weight is dependent upon the internal changes. This study focused on liver cirrhosis induced with CCL_4 and change in weight noted (Fig. 1). Liver Weights of Rat: CCl_4 and combination groups have a significant effect on liver weight of rats. The liver is being collected for chemical analysis such aslactate dehydrogenase enzyme, bilirubin total, alanine transferaseen zyme, aspartate aminotransferase, alkaline phosphatase, total protein, albumin and globulin, A/G ratio (Fig. 2).

Histopathological Investigations of Untreated Cells: The histological examination of untreated liver cells (control) revealed normal hepatic architecture. No inflammation,apoptotic bodies in hepatocytes, hydropic degeneration, fibrosis, necrosis, atypia, coagulative necrosisor malignancy were observed (Fig. 3). Histopathological features of CCL_4 treated cells: CCL_4treatedsubmittedliver indicated areas of hepatic coagulative necrosis on top of hydropic degeneration and ballooning degeneration. Hepatocytes showed cytoplasmic vacuolation and nucleus propelled towards periphery. Moderate grade mononuclear infiltration in the portal tract butno hepatic fibrosis, atypia or malignant changes (Figs. 4-5)

Histological Features of ZnO treated cells: Rats treated with glutaraldehyde exhibited areas of hepatic coagulative necrosis on top of hydropic degeneration and ballooning degeneration. Hepatocytes showed cytoplasmic vacuolation, nucleus propelled towards periphery and moderate grade mononuclear infiltration in portal tracts. Sections also showed cirrhotic changes scattered unevenly with reparative nodule. No hepatic fibrosis, atypia or malignant changes were observed (Fig. 6).

Histological Features of ZnO+CCL_4 Treated Cells: Submitted liver revealed normal hepatic architecture and vascularity. Some hepatocytes showed cytoplasmic vacuolation near to normal, nucleus was propelled towards periphery but no inflammation, fatty change, fibrosis, atypia or malignancy (Fig. 7).

Fig. 1**:** The above figure represents weight data of rats. Y-axis shows weight of rats in grams. There is drastic drop in weight of diseased rat as compared to the control rat

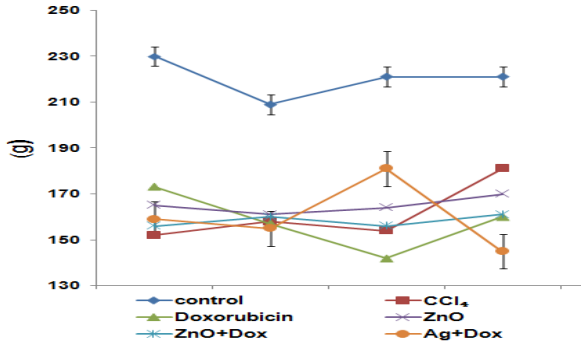


Fig. 2: Liver weight of rats. On the a-axis group of rats is indicated. On the y-axis weights are indicated

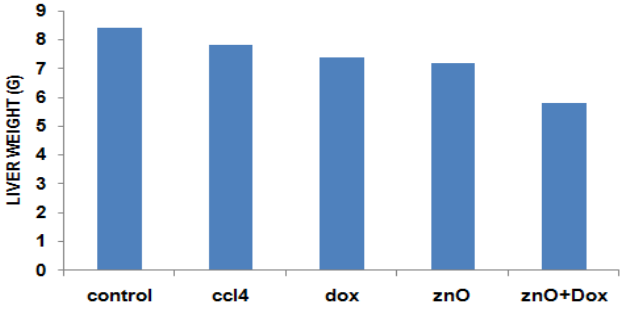


Fig. 3: Histological examination of the control rat liver showing the portal triad and the normal hepatocytes with well brought out nuclei and cytoplasm

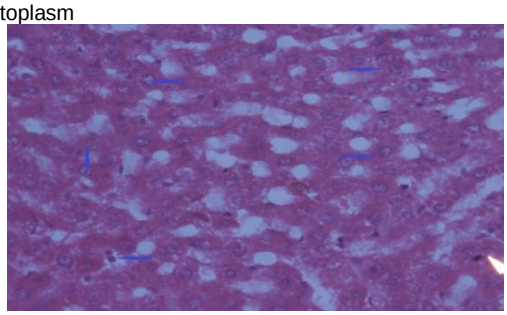


Fig. 4: Histological examination of CCl4 induced hepatotoxicity in rat liver showing macrovesicular and microvessicularsteatosis, ballooning degeneration and destruction of hepatic architecture

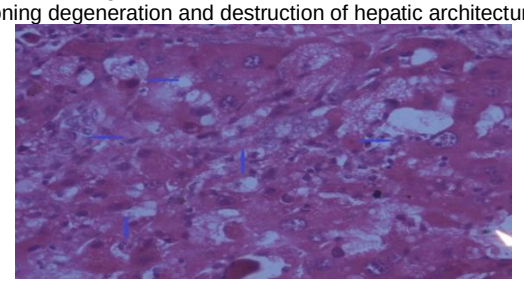


Fig. 5: Histological examination of drug induced hepatotoxicity in rat liver showing minimal hepatic damage

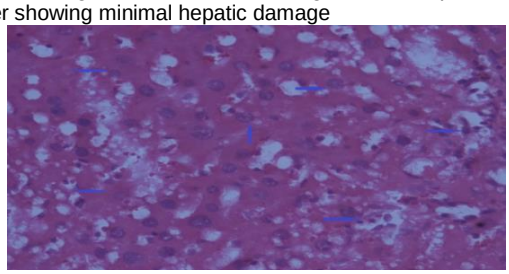


Fig. 6: Histological examination of drug induced hepatotoxicity in rat liver showing minimal hepatic damage

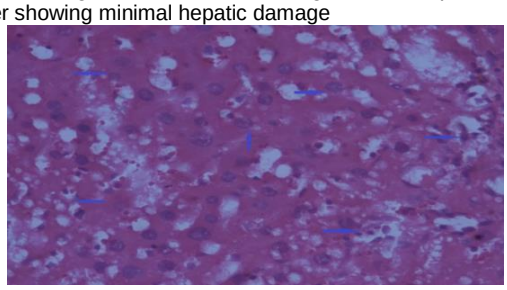
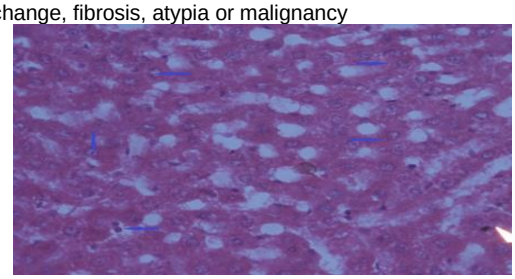


Fig. 7: Histological examination ofportal triad and normal hepatocytes with well nuclei and cytoplasmbut no inflammation, fatty change, fibrosis, atypia or malignancy



DISCUSSION

The results of current study are quite similar with some reports which showed mass of cells present on the bottom and top corners of the slides which are clearly visible in comparison with normal liver. Increase in size of cells indicated cancer group. No tumor in liver was found and best results obtained in liver function test. Liver disorder which cannot be reversed known as Liver cirrhosis was also observed (with regular scarring of tissues of liver along with nonstop regeneration). Some major factors like alcohol and viral infections, etc. are among reasons for liver cirrhosis which converts to cancer and leads todeath. There is high chance of up to 75% of tumor production with Liver cirrhosis and fibrosis which are located alongside hepatocellular carcinoma. Some viral disorders like advanced stages of hepatitis also acquire cirrhosis leading to cancer formation in later stages.[14]Fatty liver is more likely to acquire this dangerous disorder as well asmany other liver injuries are converted to cirrhosis. Deregulation of normal mechanism occur if damage is not treated for a long time and can cause deposition of proteins, fibrosis and Extracellular matrix (EXM)[15,16].

Lactate dehydrogenase test performed on rats in this study helped in identification of disease or other problems inliver. Damage to tissues can be identified with the increased levels of LDH. It was helpful to find issues with organs of rats. Results showed highLDH in rats treated with CCL_4 as compared to those treated with ZnONPs, CCL_4 and ZnONPs. Levels of LDH were close to normal range in rats treated with ZnONPs. Toxicity levels were also quite low in rats treated with ZnONPs. This study proved that there was no toxicity to normal cells when treated with ZnONPs and quite promising results against cirrhosis also observed, showingZnONPs can also be used as an anti-cirrhosis medicine.

Past study showed use of CCL_4 for the induction of liver cirrhosis in rat models. Other studies showed zinc for protection to keep the liver out of fibrosis. The properties of zinc as anti-fibrotic are still not known and studies has shown that deficiency of zinc causes the development of many disorders as well.[17]Other studies have showed that the deficiency of zinc can cause adverse effects on the normal body functioning andsevere disorders like growth retardation can be due to deficiency of zinc. Wound healing can also be delayed as it can cause neurological disorders.[18]Some of the metabolic processes are also stopped due to the unavailability of zinc in the body.

Healthy albino mice were taken for in-vivo study of ZnONPs and groups made. There were a total of five groups from which one was a control, and other three groups were treated with ZnONPs, CCl_4 and combined CCl_4 and ZnONPs. Mice treated with CCl_4 showed the presence of hepatotoxicity after 14 days which was further increased after 21 days of exposure with CCl_4. The mice which were treated with ZnONPs showed protection against toxicity due to CCl_4. Liver function close to normal were observed with ZnONPs treated group[19].

Reweighing of rats showed clearly that rats treated with CCl_4had low weight as compared to those treated with ZnONPs as well as control group. Rats were divided into three groups with one Control and other CCl_4 and ZnONPs

treated. Group treated with CCl_4 was called as diseased group and one treated with CCl_4 and ZnONPs was called as treated group. Results of gel electrophoresis and histopathology of organs clearly showed the damage to the liver caused by CCl_4. A clear toxicity was observed in liver for rats with diseased group of CCl_4. This study showed damage to the liver and increased toxicity due to CCl_4. Discoloration of spleen was also observed due to doxorubicin. This might be due to high metabolic activity of hepatocytes and there is a chance of surface damage to the cell as well. Integrity of the cell may also be lost. This may cause accumulation of fluid inside the cell. This may also cause the change of shape in the cell. This change in color and shape of the cells may be due to doxorubicin[20].

Liver function test was performed after 14 days in this study. ALT, AST, total protein, globulin, albumin, A/G ratio and bilirubin test was also conducted for all groups of rats along with control. The levels of ALT were increased in the diseased group of rats and the levels of ALT were normal for combined group of CCl_4 and ZnONPs. These were normal for untreated group as well. This may be an effect of the anti-cancer zinc particles in CCl_4 induced damage of liver. Levels of AST enzymes were increased for the group with CCl_4 which indicates towards the leakage of this enzyme by regular dose and cross hepatocellular membrane also in the blood which is main reason for the dysfunction of liver and injuries to the cells. These levels of AST were reduced in the combination group of CCl_4 and ZnONPs. Similar results were found for ALP levels and they were high in CCl_4 group of rats and reduced in combined group. High levels of ALP causes damage to the liver cells and affects the normal activity of liver. ALP is anecto-enzyme of hepatocyte plasma membrane. More injury to the liver cells was observed as well. ALP values for the combination group werecloser to normal ranges and no injury and cirrhosis was observed. There was no tumor found as well in combined group. Due to liver deferent functions, it is not possible to study function of liver with a single biochemical test and a group of test is needed to fully study the function of liver.[21]Anti-inflammatory drugs are shown to be limited in their activity even after so many attempts. The anti-fibrotic drug is available for human use against fibrosis. Treatment with zinc is specific and targeted and it effect on carbon tetrachloride in the liver of rat models.[22]Change in the biochemical serum shows that the values of ALT, AST and ALP were increased in group of CCl_4 in comparison with other healthy and control group[23].

The increase in liver enzymes levels including AST, ALT and ALP reflects damage to hepatocytes (liver cells) in diseased group[24]. High level of AST indicates injury to the hepatic cells by CCl_4 and it is released after hepatocytes damage. The increased toxicity due to CCl_4 causes increased permeability of hepatocyte membrane causing cellular leakage which leads towards raised level of ALP which is an ecto-enzyme[25]. A study showed that increased level of AST and ALT are due to repetitive dose of CCl_4 causing dysfunction of liver and injury to hepatocytes[26]. These levels of ALT, AST and ALP are reduced in treatment group after dosage of CCl_4 as compared to the diseased group which has high levels of these enzymes along with cellular injury[23]. Similar study reported was also proving these results.[27]

ZnONPs may produce some toxicity in the cells. Accumulation of some cells around portal point and portal vein can also be observed along with inflammation, may be due to release of zinc in ionic state which leads towards production of reactive oxygen species (ROS). Activity of p53 gene is also observed. This gene gets activated due to damage to the DNA or any instability inside DNA. Apoptosis is caused by this p53 gene. This gene activation may be due to ZnONPs which needs to be clarified in further studies[28,29]. Cancerous cells are affected by ZnONPs and can be killed by these nanoparticles. Studies have showed that cancer cells can be killed by ZnONPs with least side effects to other cells. Toxicity linked with these nanoparticles is based on concentrations used. These nanoparticles are selective in nature and target only specific cells which can further be enhanced by the use of probes. They are designed specifically for targeted cells with least effects on the normal cells[30,31].They have ability to kill cancer cells specifically and can be used as targeted therapeutic medicine for cancer cells.[32,33]This study clearly showed that there was no damage or toxicity to other organs by the use of ZnONPs.

CONCLUSION

Nanoparticles which are biologically synthesized even by low budget showed excellent results in current study. ZnONPs nanoparticles have ability to induce apoptosis as well as release of reactive oxygen species (ROS) in cells. This is the reason why ZnONPs is considered as a great nanoparticle against cancer and microbes. Results showed that ZnONPs tends to have a very low damage to liver in the presence of a toxic material (Carbon tetrachloride) which results in liver cirrhosis if not treated. Rats which were treated with CCl_4has a considerable low weight in comparison to those treated with ZnONPs and this was due to liver cirrhosis in rats treated with CCl_4. Blood test such as Lactate dehydrogenase enzyme and liver function test results of rats treated with CCl_4 also showed presence of disease and damage to the liver. Whereas in case of rats treated with ZnONPs showed remarkable results in controlling disease and less damage to the liver. ZnONPs has shown to be quite promising in treatment against cancer and microbes but have some issues of toxicity. Deposition of zinc in vital organs is a real problem as it can cause some major problems in long range. Nanoparticles like ZnONPs has a great future in medical field and these can also be made target specific by combining with aptamers. ZnONPs and other nanoparticles can be used directly against cancer cells and this way healthy cells can be kept safe.Nanoparticles has a problem of toxicity which is the only problem with them, which can be covered with the help of probes that can bind with these nanoparticles and help in detection of specific cells having high amounts of surviving protein present abundantly in cancer cells.This way cancer cells can be killed directly. Future of medicines has a major role of nanoparticles like ZnONPs. Experimentalanimal model used in this study can a good source of treating the development of liver cancer in its early stage with the help of ZnONPs with least side effects.

REFERENCES

1. MohsenH, El-Dahshan ES, El-Horbaty ES, Salem AB. Classification using deep learning neural networks for brain tumors.Future Computing and Inform J2018; 3(1):68-71.
2. GalonJ, PagèsF, MarincolaFM, Angell HK, ThurinM, Lugl iA, et al. Cancer classification using the Immunoscore: a worldwide task force.JTranslatMed2012;10(1):1-0.
3. QuerM, Vander PoortenV, Takes RP, Silver CE, Boedeker CC, de Bree AR, et al. Surgical options in benign parotid tumors: a proposal for classification. Eur Arch Otorhinol2017; 274(11):3825-36.
4. Taghizadeh S, Alimardani V, Roudbali PL, Ghasemi Y, Kaviani E. Gold nanoparticles application in liver cancer. Photodiagn Photodynther 2019.
5. FukuiH, Saito H, Ueno Y, UtoH, Obara K, SakaidaI, et al. Evidence-based clinical practice guidelines for liver cirrhosis 2015.J Gastroenterol2016;51(7):629-50.
6. TsochatzisEA, BoschJ, BurroughsAK.Liver cirrhosis. Lancet 014; 383(9930):1749-61.
7. Kahn AM, Ozeran RS. Liver and serum zinc abnormalities in rats with cirrhosis.Gastroenterology 1967;53(2):193-7.
8. Kołodziejczak-Radzimska A, Jesionowski T. Zinc oxide - from synthesis to application: a review.Mater2014;7(4):2833-81.
9. PauziN, ZainNM,YusofNA. Microwave-assisted synthesis for environmentally ZnO nanoparticle synthesis. InProceedings of the 10th National Technical Seminar on Underwater System Technology 2018Springer, Singapore2019; pp 541-6.
10. Shobha N, Nanda N, Giresha AS, Manjappa P, Sophiya P, Dharmappa KK, et al. Synthesis and characterization of Zinc oxide nanoparticles utilizing seed source of Ricinuscommunis and study of its antioxidant, antifungal and anticancer activity.Mater SciEngColl2019;97:842-50.
11. Pullagurala VL, AdisaI O, Rawat S, KimB, Barrios AC, Medina-VeloI A, et al. Finding the conditions for the beneficial use of ZnO nanoparticles towards plants-A review. Environ Pollut 2018; 241:1175-81.
12. Lopes S, Ribeiro F, Wojnarowicz J, Łojkowski W, Jurkschat K, Crossley A, et al. Zinc oxide nanoparticles toxicity to Daphnia magna: size-dependent effects and dissolution. Environ ToxicolChem2014;33(1):190-8.
13. He E, Qiu H, Huang X, Van Geste lCA, Qiu R. Different dynamic accumulation and toxicity of ZnO nanoparticles and ionic Zn in the soil sentinel organism Enchytraeuscrypticus. Environ Poll2019;245:510-8.
14. Ijuin H. Evaluation of pancreatic exocrine function and zinc absorption in alcoholism. Kurume Med J 1998;45(1):1-5.
15. Tsochatzis EA, Bosch J, Burroughs AK. Liver cirrhosis. Lancet 2014;383(9930):1749-61.
16. Martinez EE, Shimoda W. Determination of monensin sodium residues in beef liver tissue by liquid chromatography of a fluorescent derivative. JAOAC 1985;68(6):1149-53.
17. Prasad PN, Bergey EJ, Liebow C, Levy L, inventors; Research Foundation of State University of New York, assignee. Magnetic nanoparticles for selective therapy. United States patent US 6,514,481. 2003.
18. Teng X, Black D, Watkins NJ, Gao Y, Yang H. Platinum-maghemite core-shell nanoparticles using a sequential synthesis. Nano Lett 2003;3(2):261-4.
19. Jiménez-Sánchez M, Pérez-Morales R, Goycoolea FM, Mueller M, Praznik W, Loeppert R, et al. Self-assembled high molecular weight inulin nanoparticles: Enzymatic synthesis, physicochemical and biological properties. Carbohydpolym 2019;215:160-9.
20. Shiha G, Sarin SK, Ibrahim AE, Omata M, Kumar A, Lesmana LA, et al. Liver fibrosis: consensus recommendations of the Asian Pacific Association for the Study of the Liver (APASL). HepatolInt2009;3(2):323-33.
21. Thapa BR, Walia A. Liver function tests and their interpretation. The Ind J Pediat 2007;74(7):663-71.
22. Anttinen H, Ryhänen L, Puistola U, Arranto A, Oikarinen A. Decrease in liver collagen accumulation in carbon tetrachloride-injured and normal growing rats upon administration of zinc. Gastroenterology 1984;86(3):532-9.
23. Sourianarayanane A, Garg G, Smith TH, Butt MI, McCullough AJ, Shen B. Risk factors of non-alcoholic fatty liver disease in patients with inflammatory bowel disease. J Crohn's Colitis 2013;7(8):279-85.
24. Wolf PL. Biochemical diagnosis of liver disease. Ind J ClinBiochem 1999;14(1):59-90.
25. Paduraru I, Saramet A, Danila GH, Nichifor M, Jerca L, Iacobovici A, et al. Antioxidant action of a new flavonic derivative in acute carbon tetrachloride intoxication. EurJ Drug MetabPharmacokine 1996;21(1):1-6.
26. Muriel P, Escobar Y. Kupffer cells are responsible for liver cirrhosis induced by carbon tetrachloride. Journal of Applied Toxicology: Int J 2003;23(2):103-8.
27. Singh S, Fujii LL, Murad MH, Wang Z, Asrani SK, Ehman RL, et al. Liver stiffness is associated with risk of decompensation, liver cancer, and death in patients with chronic liver diseases: a systematic review and meta-analysis. ClinGastroenterolHepatol 2013;11(12):1573-84.
28. Farnebo S, Winbladh A, Zettersten EK, Sandström P, Gullstrand P, Samuelsson A, et al. Urea clearance: a new technique based on microdialysis to assess liver blood flow studied in a pig model of ischemia/reperfusion. EurSurg Res 2010;45(2):105-12.
29. Burgess SC, Hausler N, Merritt M, Jeffrey FM, Storey C, Milde A, et al. Impaired tricarboxylic acid cycle activity in mouse livers lacking cytosolic phosphoenolpyruvate carboxykinase. J BiolChem 2004;279(47):48941-9.
30. Ahmad J, Ahamed M, Akhtar MJ, Alrokayan SA, Siddiqui MA, Musarrat J, et al. Apoptosis induction by silica nanoparticles mediated through reactive oxygen species in human liver cell line HepG2. Toxicol Applpharmacol 2012;259(2):160-8.
31. Hanley C, Layne J, Punnoose A, Reddy KÁ, Coombs I, Coombs A, et al. Preferential killing of cancer cells and activated human T cells using ZnO nanoparticles. Nanotechn 2008;19(29):295103.
32. Hu X, Cook S, Wang P, Hwang HM. In vitro evaluation of cytotoxicity of engineered metal oxide nanoparticles. Sci Total Environ 2009;407(8):3070-2.
33. Eroğlu İ, Azizoğlu E, Özyazıcı M, Nenni M, Gürer Orhan H, Özbal S, et al. Effective topical delivery systems for corticosteroids: dermatological and histological evaluations. Drug Deliv 2016;23(5):1502-13.