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Haloperidol-Loaded Chitosan Nanocomposites Improve Liver and Kidney Functions and Lipid Profile of Male Rats.

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ABSTRACT

Haloperidol, widely used standard neuroleptic drug, chronic use is linked to development of tardive dyskinesia and metabolic disturbance. So that this work aims to overcome haloperidol side effects by using chitosan nanoparticles as poly load system for this drug. Chitosan nanoparticles we prepared and characterized followed by loading haloperidol drug on chitosan nanoparticles. IR spectroscopy and transmission electron microscope (TEM) were used to to characterize these nanoparticles. The IR and TEM analysis were confirmed that there are conjugation between these nanoparticles and the haloperidol drug. The effect of haloperidol-chitosan nanocomposites on liver functions, kidney functions and lipid profiles were evaluated compared with the effect of haloperidol drug alone. In the *in vivo* study, the rats were divided into six main groups as follows, control, haloperidol low dose (0.3 mg/kg bwt/day), haloperidol high dose (1 mg/kg bwt/day), nano group treated with the nanoparticles alone, haloperidol nanoparticles low dose and haloperidol nanoparticles high dose given the same low and high doses of haloperidol to the rats. All the groups treated for 21 days before decapitation and collecting the blood for assessment of serum alanine transaminase, aspartate transaminase, alkaline phosphatase, total proteins, Creatinine, urea, total cholesterol, triglycerides, high density lipoproteins, low density lipoproteins, and very low density lipoproteins. The results revealed improvement in the liver, kidney functions and lipid profile in the haloperidol nanoparticles loaded groups in comparison with the treatment with low and high dose of haloperidol alone. The present results concluded that the haloperidol loaded on chitosan nanoparticles has fewer side effects on liver, kidney and lipid profile and could be used as treatment for schizophrenia, manic states and neurological disorders instead of haloperidol alone.

Keywords: haloperidol, poly loaded chitosan nanoparticles, nanocomposites, liver and kidney functions and lipid profile

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INTRODUCTION

Haloperidol (H) is a standard or typical neuroleptic drug. It is broadly used for the treatment of schizophrenia, delusions and hallucinations. The pharmacological action of it includes the blockage of D2 receptors (Bernard and Simona, 2005). However, haloperidol chronic use is linked with the progress of tardive dyskinesia (TD) of the patients (Kane and Smith, 1982; Gaertner et al., 2001) and its occurrence raises powerfully with age (Yassa and Jeste, 1992). The abnormal and involuntary movements of the orofacial area is characterized the disease, and occasionally, trunk and members musculature can show through treatment or after withdrawal of with neuroleptic drugs (Kane, 1995). It has been found that haloperidol induce also metabolic disturbances, involving weight gain (Bobes et al., 2003; Zipursky et al., 2005), hyperglycemia (Wirshing et al., 2002; Perez-Iglesias et al., 2009), insulin resistance (Perez-Iglesias et al., 2009), and a deterioration of the lipid profile (Perez-Iglesias et al., 2009).

Haloperidol is treatment for schizophrenia, manic states, neurological disorders with hyperkinesias (Yasir and Sara, 2014). The oral pathways of the drug result in only a small fraction being able to arrive at the brain from the blood (Vella-Brincat and Macleod, 2004). The high systemic concentrations of haloperidol involve respiratory disturbance, nausea, vomiting, dermatological reactions and musculoskeletal disorder (Budhian et al., 2007). Consequently, a drug delivery system is necessary, which gives fast and marked delivery to brain and also decreases the systemic exposure (Kaur et al., 2008). The antipsychotic drugs (APDs) loaded with nanoparticles have been examined in rodent models using different routes of administration to treat schizophrenia (Kumar et al., 2008; Singh and Lillard, 2009).

Chitosan is a copolymer of N-acetyl glucose amine and glucose amine monomer units (Chattopadhyay and Inamdar, 2013). It can be used as anti-bacterial and anti-fungal material due to its biocompatibility, biodegradability and non toxic material (Avadi et al., 2004; Abou-Zeid et al., 2011).

Chitosan nanoparticles used as delivery systems in several previous studies (Ibrahim et al., 2015), such as insulin to enhance its absorption to maximize its bioavailability (Pan et al., 2002). In addition chitosan nanoparticles used as gene carrier to enhance the efficiency of gene transfer in cells (Qi et al., 2004).

The main goal of this work is minimized the undesirable side effects of haloperidol by using chitosan nanoparticles as poly load system for this drug. Firstly we prepared and characterized chitosan nanoparticles. Secondly, load the haloperidol drug on chitosan nanoparticles. IR spectroscopy and transmission electron microscope (TEM) studies to characterize these nanoparticles. Finally, evaluate the haloperidol-chitosan nanocomposites effect on liver functions, kidney functions and lipid profiles in comparison with the haloperidol alone.

MATERIALS AND METHODS

Materials

Chitosan (Alfa Aesar Company, Medium molecular weight, viscosity 1860 cps, degree of deacetylation 79.0%), penta sodium tri poly phosphate (TPP). Sodium hydroxide (Modern Lab chemicals, Egypt), Methyl alcohol, ethyl alcohol and acetic acid (Sisco Research Laboratories, India) and all other chemicals used are analytical grade and were used without further purification.

Methods:

Preparation of chitosan nanoparticles

Chitosan nanoparticles were prepared based on the modified ionotropic gelation(Ibrahim et al., 2015). Briefly, Chitosan was dissolved in 1% (v/v) acetic acid and leaving it under stirring for 24 hr. The pH was adjusted to pH 5.5 with 0.01N NaOH. TPP was dissolved separately in deionized water to final concentration of 0.1 mg/ml. Then, the TPP solution was added to the chitosan solution drop wise at different TPP:chitosan ratios under vigorous magnetic stirring at room temperature. The resulting suspension was then left under Ultrasoincation for 45 min.

Preparation of Haloperidol-loaded chitosan nanoparticles:

Different concentration of the antibiotic dissolved in distilled water was added to nano chitosan solution in the same molar ratio with stirring for 20 min. and the resulting suspension was then left under ultra-sonication for 45 min. then finally stirring for another 20 min., to obtain a final haloperidol concentration (0.05, 0.1, .15, 0.2, 0.5% mg/ml).

Characterization of Chitosan Nano particle and its loaded Drug:

- The FTIR spectra of the samples were recorded by using an FT- IR spectrophotometer (Nexus 670, Nicolet, USA) in the region of 4000-400cm-1 with spectra resolution of 4 cm-1.
- Shape and size of chitosan Nano particle was investigated using JEOL, TEM-Specimens for TEM measurements were prepared by placing a drop of colloidal solution on 400 mesh copper grid coated by an amorphous carbon film and evaporating the solvent in air at room temperature. The average diameter of chitosan Nano particle was determined from diameter of 100 nano particle found in several arbitrarily chosen are in enlarged microphotographs.

In vivo study:

Experimental animals:

Forty-eight adult male albino rats (Wistar strain) were used in this study (150g ±10g). The rats obtained from the animal house of National Organization for Drug Control and Research (NODCAR). Rats housed in iron mesh cages with eight rats each. The experimental animals allowed acclimating under the laboratory conditions two weeks before the beginning of the experiments and kept under controlled temperature of 25^0C±2 and 12 hours' light / dark cycle throughout the experiment. Animals feed on a commercial pelleted diet. The animal care conforms to the Guide for the Care and Use of Laboratory Animals of Ain Shams University, which was in accordance with UK, Animals (Scientific Procedures) Act, 1986 and the European Communities Council Directive, associated guidelines, of 24 November 1986 (86/609/EEC) (COUNCIL, 2005).

Drugs:

Haloperidol decanoate, $C_{21}H_{23}ClFNO_2$, phenyl - piperdinyl - butyrophenone, was manufactured by Sunny pharmaceutical and packed by Marcyrl pharmaceutical and industries, Egypt, as ampoules (5mg/1ml) for muscle injection.

Experimental groups:

The rats were divided into six groups as follows:

1.Control group (Con): administered via intraperitoneal saline as 2 ml/ kg b.wt./ day for three weeks.
2.Low dose haloperidol group (HLD): administered via intraperitoneal haloperidol as 0.3mg / kg b.wt./ day (Baptista et al., 2013) for three weeks. The dose was dissolved in the same amount of vehicle.
3.High dose haloperidol group (HHD): administered via intraperitoneal haloperidol as 1 mg / kg b.wt./ day **(Adedeji et al., 2014)** for three weeks. The dose was dissolved in the same amount of vehicle.
4.Control nano group (Nano): administered via intraperitoneal nanoparticles alone dissolved in distilled water as 2 ml/ kg b.wt./ day for three weeks.
5.Low dose haloperidol loaded with nanoparticles group (HLD nano): administered via intraperitoneal haloperidol as 0.3mg / kg b.wt./ day for three weeks carried in the same amount of nanoparticles. The dose was dissolved in the same amount of vehicle (distilled H2O).
6.High dose haloperidol loaded with nanoparticles group (HHD nano): administered via intraperitoneal haloperidol as 1 mg / kg b.wt./ day for three weeks carried in the same amount of nanoparticles. The dose was dissolved in the same amount of vehicle (distilled H2O).

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Biochemical assays:

At the end of the experimental periods 21 days, animals sacrificed after 24 hours from the last administration dose by rapid decapitation. Blood samples collected and serum obtained and stored at -80 for biochemical analysis. Serum aspartate aminotransferase (AST, GOT) and alanine aminotransferase (ALT, GPT) enzymes were determined calorimetrically according to reported method (Schumann et al., 2002). The alkaline phosphatase activity was determined by Kinetic photometric test using DiaSys Diagnostic kit, creatinine was detected using Spin react kit and serum urea was assayed enzymatically using DiaSys Diagnostic kit. In addition, serum cholesterol (Young and Friedman, 2001), triglycerides (TG) (Fossati and Prencipe, 1982), HDL, LDL and VLDL were determined colorimetrically using DiaSys Diagnostic kits.

Statistical Analysis:

Reported values represent means of 8 rats ± SE. Statistical analysis evaluated by one-way ANOVA. Once a significant F test obtained, LSD comparisons performed to assess the significance of differences among various treatment groups. Social package "SPSS" for Windows software, Release 18.0 (SPSS, Chicago,IL) was used.

RESULTS AND DISCUSSION

There are number of NH_2 groups in chitosan backbone which converted into NH_3^+ in acidic medium so that it can be physical and chemical cross-linked and used to prepare nanoparticles (Kafshgari et al., 2011). There are advantage of using physical cross-lining than chemical cross-linked such as no toxic materials found and minimized side effects to improve its biocompatibility (Agnihotri et al., 2004; Rayment and Butler, 2008). Physical cross-linking is formed from complexes of chitosan positive charges and multivalent ions negative charges e.g. sodium tripolyphosphate (TPP) (Bodmeier et al., 1989), citrate, and sulfate (Shu and Zhu, 2001).

Preparation and Characterization of chitosan nanoparticles:

Ionic gelation method used to prepare chitosan nanoparticles (SNPs) via ionic forces between positive charges on chitosan and negative charges on Penta sodium tri phosphate (TPP) at ordinary temperatures (Rampino et al., 2013; Ibrahim et al., 2015; Wijesena et al., 2015). our previous studies concluded that the optimum conditions for preparation of well distributed chitosan nanoparticles were 0.2 g\ml chitosan solution concentration, 0.1 g\ml TPP concentration, 5.5 pH and 45 min ultra-sonication time(Ibrahim et al., 2015). At these optimum conditions nanoparticles began because inter and intra molecular cross linkage between chitosan and TPP molecules formed via anionic molecules (Xu and Du, 2003; Rampino et al., 2013)

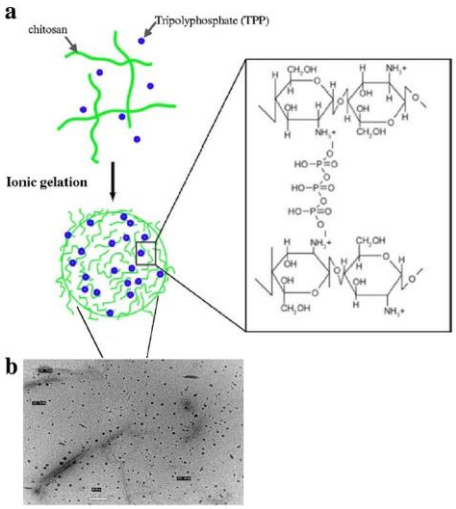

Figure 1: Chitosan nanoparticles-tripolyphosphate complex by ionic gelation with TEM image of CSNPS with 25 nm diameter at optimum condition

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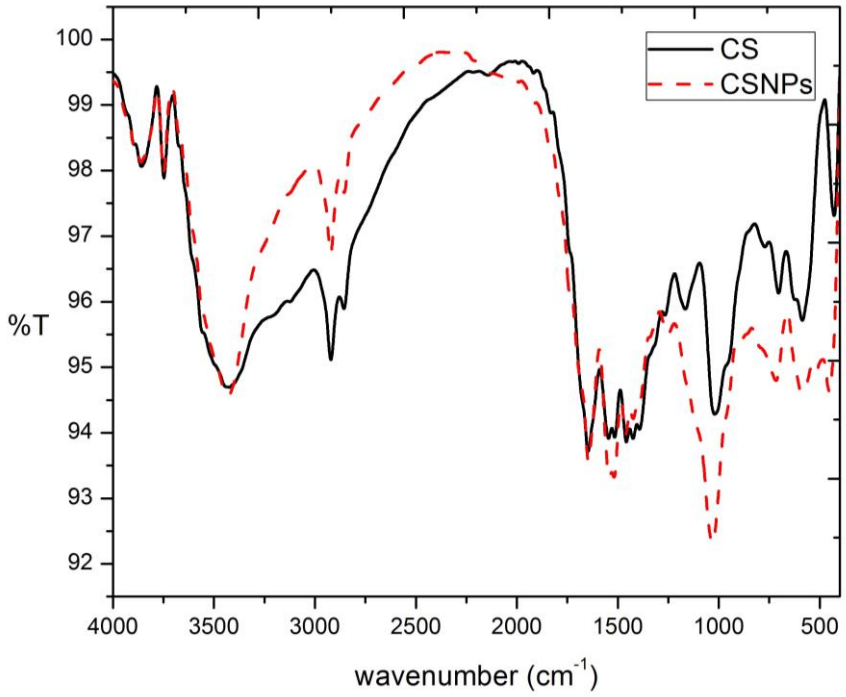

Figure 2: FT-IR of native chitosan (CS) and chitosan nanoparticles (CSNPs)

The interaction between chains of chitosan and TPP was determined by using FT-IR spectra of native chitosan (CS) and prepared chitosan nanoparticles (CSMPs). These results were similar to previous data published (Dudhani and Kosaraju, 2010). There band peak between 3350 cm^{-1} and 3300 cm^{-1} corresponding to mix stretching of OH and NH bonds in chitosan molecule which become wider and shifts to lower wave number values for H. bonding interactions (Jia-hui et al., 1999). Band for bending vibrations of amino group at 1523 cm^{-1} for chitosan shifted to 1533 cm^{-1} for chitosan nanoparticles as found in CSNPs-TPP films (Jia-hui et al., 1999) which due to interaction of amino groups with sodium poly phosphate anions (Knaul et al., 1999). The bands observed at 1201 and 885cm^{-1} corresponding to P-O stretching and bending in TPP respectively (Xu and Du, 2003).

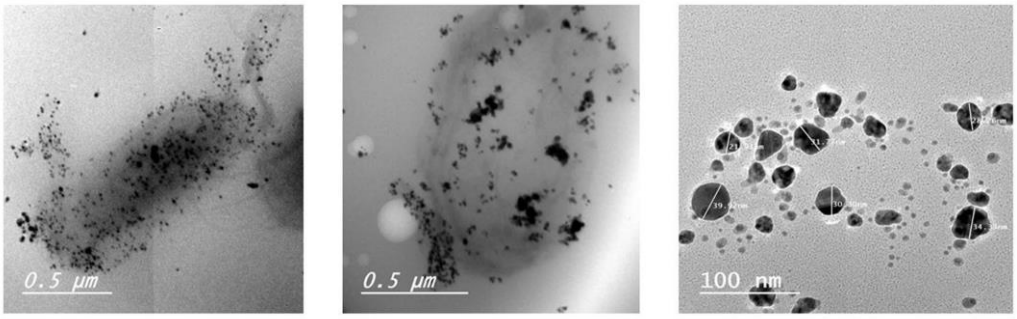

Figure 3: TEM images of the prepared optimized CSNPs at 5.5 pH for 45 min sonication time

TEM images confirm that we obtain small spheres and individual uniform CSNPs with diameter within 15-25 nm. The presence of large particles comes from some type of aggregation of single small particles which gathered together to form these large particles, these aggregation increased as the concentration of CS and TPP solutions increased (Ibrahim et al., 2015).

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Haloperidol-loaded chitosan nanocomposites properties:

In the present work, the prepared chitosan nanoparticles used to coat haloperidol drug to obtain haloperidol delivery system with dimensioned adverse side effects. Figure 4 shows the TEM images of nanocomposites with haloperidol compared with the images of chitosan nanoparticles alone as showed in figure 3. Figure 4 shows the incorporation of haloperidol into chitosan nanoparticles as ploy load system due to some types of intermolecular hydrogen bonds between NH_3^+ and electronegative charges in haloperidol to form semi-network (Xu and Du, 2003). So that TEM images prove that haloperidol drug attached to chitosan nanoparticles surface.

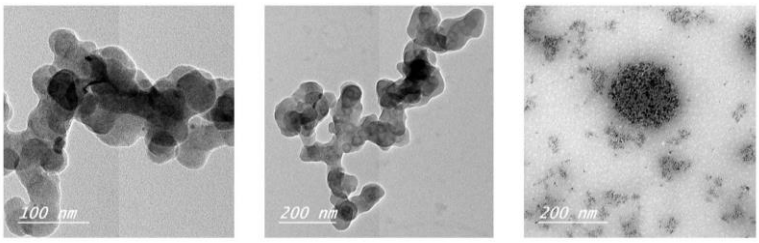

Figure 4: TEM images of haloperidol loaded chitosan nanoparticles

In vivo study:

In the present study (Figure 5) showed that significant increases in the Alanine transaminase (ALT), aspartate transaminase AST and alkaline phosphatase ALP levels in low and high doses of haloperidol ($p<0.05$) in comparison with the control, while the nano, low dose of haloperidol loaded nanoparticles and high dose of haloperidol loaded nanoparticles groups (HLD nano and HHD nano) exhibited a significant decrease in ALT, AST and ALP activities in comparison with HLD and HHD groups. Liver injury or Liver dysfunction occurs by drugs, appears when there is an increase of more than 2 times the normal range of ALT or a total elevation in AST and ALP. In this case the used drug should be withdrawn (O'Donnell et al., 2014). In the Popovic et al. study (Popovic et al., 2008), showed that haloperidol treatment and alcohol oxidative stress activities liver damage via the liver ALT activities. Whereas, Atasoy et al. (Atasoy et al., 2007) studies demonstrated that haloperidol, as typical antipsychotics (APs) drugs, may cause liver toxicity while, atypical APs may regulate liver enzyme functions. Furthermore, Haduch et al. (Haduch et al., 2005; Haduch et al., 2005; Haduch et al., 2007) revealed that haloperidol decrease the CYP450 isozymes activity in the rat livers. Wei et al. (Wei et al., 2009) reported that the mitochondrial function contributes to the toxic profile of haloperidol. Andreazza et al. (Andreazza et al., 2015) revealed that haloperidol may cause oxidative stress in brain and liver, related with the documented adverse effects of the drug.

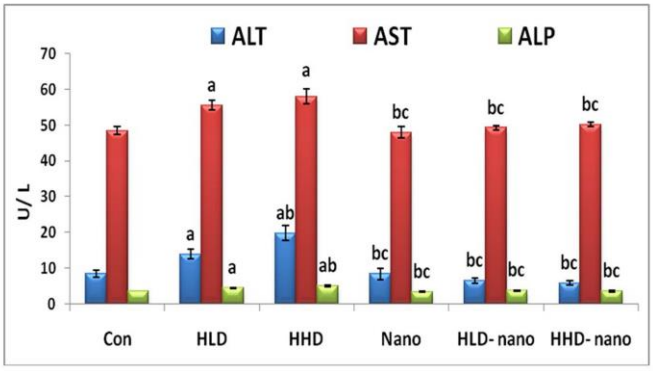

Figure 5: the serum alanine transaminase GPT (ALT), aspartate transaminase GOT (AST) and alkaline phosphatase ALP activities in different treated groups. The data presented as means ± SE of eight rats. a= significant from control, b= significant from HLD (haloperidol low dose), c= significant from HHD (haloperidol high dose), d= significant from Nano (nanoparticles group) and e= significant from HLD-nano (haloperidol low dose loaded on nanoparticles).

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The data depicted in figure 6 revealed a significant increase in serum total proteins in HLD and HHD groups in comparison with the control. While the nano, HLD nano and HHD nano rats showed a significant decrease in serum total proteins contents as compared with the HHD group. Moreover, significant decreases were shown in the nano group in serum total proteins when compared with the HLD and HHD rats at $p<0.05$. In contrast to the reported results (Roversi et al., 2015) showed that haloperidol treatment at dose (0.5 mg / kg ip) caused no significant change in plasma total protein contents. Procyshyn et al. (Procyshyn et al., 2003) showed that haloperidol leaving the lipoprotein-deficient fraction were it is primarily bound to albumin and a α -acid glycoprotein which caused redistribution. This redistribution of haloperidol may influence its clinical efficacy.

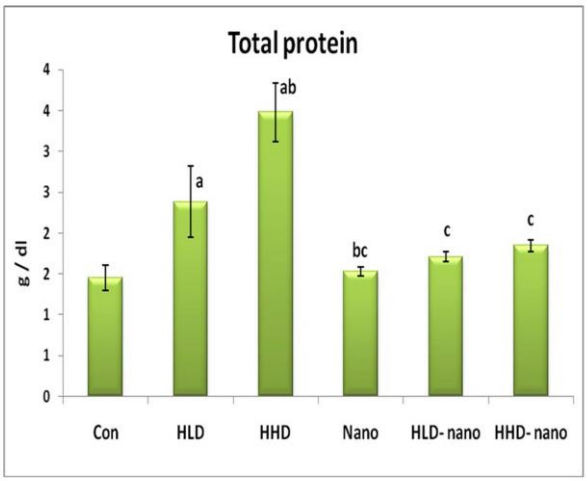

Figure 6: the serum total proteins level in different treated groups. The data presented as means ± SE of eight rats. a= significant from control, b= significant from HLD (haloperidol low dose), c= significant from HHD (haloperidol high dose), d= significant from Nano (nanoparticles group) and e= significant from HLD-nano (haloperidol low dose loaded on nanoparticles).

The data presented in figure 7 showed a significant increase in serum creatinine in haloperidol groups (HLD and HHD) in comparison with the control at $p<0.05$. The nano group showed a significant decrease in serum creatinine in comparison with HLD and HHD group, while the HLD nano and HHD nano rats exhibited a significant decrease in comparison with the HHD rats. Furthermore, the high dose of haloperidol (HHD) exhibited a significant increase in serum urea level in comparison with the control. While the nano, HLD nano and HHD nano group exhibited a significant decrease in comparison with HHD group. The increase in urea and creatinine levels could be explained by Kang et al. (Kang et al., 2006) who showed that two haloperidol neurotoxic pyridinium metabolites, 4-(4-chlorophenyl)- 1-[4-(4-fluorophenyl)-4-oxybutyl]pyridinium ion (HPP^+) and 4-(4-(chlorophenyl)-1–4-(fluorophenyl)-4-hydroxybutyl-pyridinium ($RHPP^+$), are produced in the liver and found in the brain. Haloperidol can be bio-transformed into toxic metabolites by the action of cytochrome P450 3A4, which is a major hepatic drug metabolizing enzyme. The haloperidol metabolites can be eliminated in the urine which is highly expressed in the kidney. In the same line with the present study, Roversi et al. (Roversi et al., 2015) showed that haloperidol treatment at dose (0.5 mg / kg ip) induced significant increases in AST, ALT in comparison with control and non significant increase in the serum creatinine and urea in comparison with the control. Also, the loaded nanoparticles in his work, improve the results. In the present study, haloperidol loaded nanoparticles showed improvement in the liver and kidney functions which suggest a new way to alleviate the haloperidol side effects on liver.

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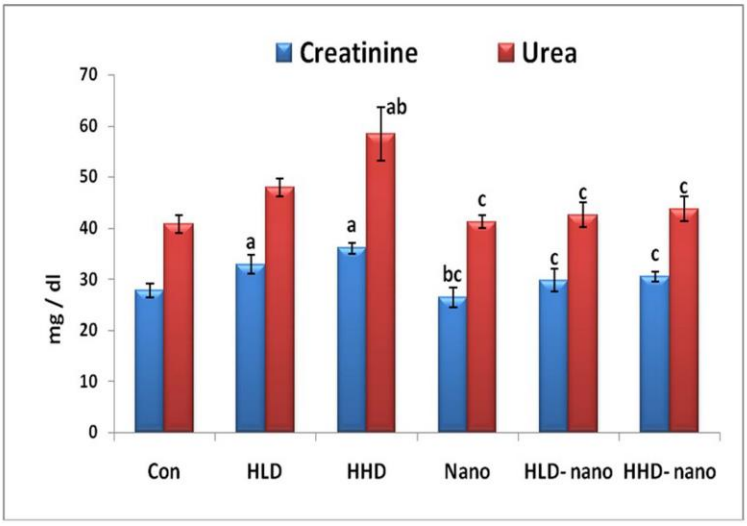

Figure 7: the serum creatinine and serum urea levels in different treated groups. The data presented as means ± SE of eight rats. a= significant from control, b= significant from HLD (haloperidol low dose), c= significant from HHD (haloperidol high dose), d= significant from Nano (nanoparticles group), e= significant from HLD-nano (haloperidol low dose loaded on nanoparticles).

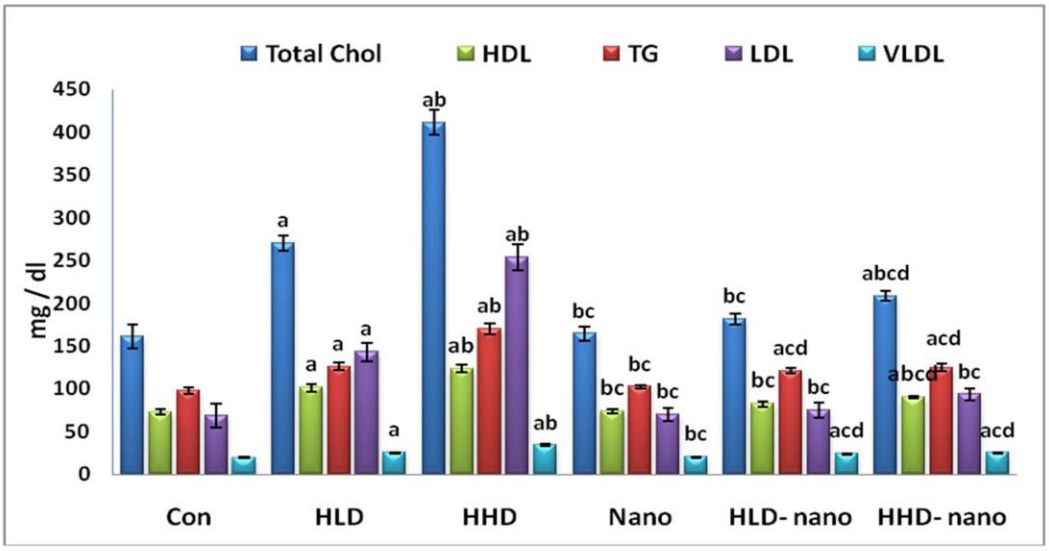

Figure 8: the serum total cholesterol (total Chol), high density lipoproteins (HDL), triglycerides (TG), low density lipoproteins (LDL) and very low density lipoproteins (VLDL) levels in different treated groups. The data presented as means ± SE of eight rats. a= significant from control, b= significant from HLD (haloperidol low dose), c= significant from HHD (haloperidol high dose), d= significant from Nano (nanoparticles group) and e= significant from HLD nano (haloperidol low dose loaded on nanoparticles).

Figure 8 illustrated that the HLD and HHD treated groups exhibited a significant increase in serum total cholesterol level, high density lipoprotein HDL, triglycerides TG, low density lipoprotein LDL, and very low density lipoprotein VLDL levels in comparison with the control or nano group at $p<0.05$. Furthermore, the HLD nano rats showed a significant increase in the serum TG and VLDL levels as compared to the control one. However, the HHD nano rats exhibited significant increases in serum total cholesterol, HDL, TG and VLDL levels in comparison with the control group. Moreover, the HLD nano and HHD nano rats showed significant

decreases in the investigated parameters in comparison with the HLD or HHD rats. While, the HLD nano rats exhibited significant increases in serum TG and VLDL levels in comparison with the Nano group. Moreover, the HHD nano rats exhibited significant increases in the investigated parameters except the LDL level in comparison with the Nano group. Haloperidol increased the plasma 18:1/18:0 ratio (desaturation index), plasma TG levels and poly unsaturated fatty acids in male rats (McNamara et al., 2011). Polymeropoulos et al. (Polymeropoulos et al., 2009) showed the effect of antipsychotics (haloperidol) on the biosynthesis and regulation of fatty acids and cholesterol; they discussed the lipid hypothesis states that the changes in lipid homeostasis might induce the schizophrenia pathogenesis. The side effects of haloperidol include metabolic disturbances, weight gain and insulin resistance (Perez-Iglesias et al., 2009). The distraction of lipid profile is due to the hinder of biosynthesis of cholesterol and interference in intracellular cholesterol transport (Sanchez-Wandelmer et al., 2010). Krakowski and Czobor (Krakowski and Czobor, 2011) found association between change in cholesterol and alternation of cognition in haloperidol group. Ikemura et al. (Ikemura et al., 2012) reported that haloperidol dosage is inverse proportion with its blood concentration in individuals with severe fatty liver disease than in those with normal livers. The haloperidol loaded with nano groups improved the lipid profile although it still has significant increase in comparison with the control.

CONCLUSION

Chitosan nanoparticles (CSNPs) was prepared and characterized by using FT-IR and TEM. These nanoparticles were used as poly load of haloperidol drug to overcome its side effects. The study results showed that haloperidol loaded chitosan nanoparticles has less side effect on liver and kidney functions accompanied by improvement of lipid profile in comparison with the haloperidol alone in both low and high doses.

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