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Original Research

The evaluation of toxicological effects of cinnamon nanoparticles by histopathological examination

Toxicological effects of cinnamon nanoparticles

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Abstract

Aim: The aim of the present study is to assess the potential toxic effects of the different doses of nanoparticles of cinnamon on the liver and kidney of mice .
Materials and Methods: After the preparation of nanoparticles of cinnamon, the three experimental groups (A1, A2, and A3) of the animals were fed 5 ppm, 10 ppm, and 20 ppm respectively while the control group (B) was not exposed to cinnamon nanoparticles. Each group of animals contains six BALB/c mice. Liver and kidney tissue samples from the experimental groups and control group were evaluated for possible toxicological effects by histopathological examination.
Results: The histological examination of liver revealed mild inflammation in Group A3 that was fed 20ppm of nanoparticles of cinnamon while the other experimental groups (A1, A2) and control group showed no morphological evidence of toxic effects. The histopathological assessment of kidneys specimen of the animals from the experimental Group A3 showed vacuolization in the tubules while no morphological alterations were seen in other experimental groups and the control group.
Discussion: Cinnamon nanoparticles showed some histological evidence of toxic effects in the liver and kidney of rats with their higher dosage.

Keywords
Cinnamon; Nanoparticles; Toxicity

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Introduction

Cinnamon is a quite delicious spice which is used as a flavoring substance in a variety of foods. The source of cinnamon is bark of trees belonging to the genus cinnamomum which is quite prevalent in Indonesia and China. Over eighty different compounds have been extracted from the cinnamon. The volatile oils derived from the cinnamon revealed antioxidant properties [1]. Cinnamomum zeylanicum exhibited properties which have healthy effects on cardiovascular system and beneficial effect on blood glucose level [2]. The use of cinnamon polyphenols has been associated with reduction in the body weight and lowering of visceral fat. The use of cinnamon is also associated with a reduced level of triglycerides and cholesterol [3]. These also reduce the oxidative stress and inflammation [4]. Certain studies revealed that the cinnamon reduces the serum C- reactive protein (CRP) which is an acute-phase protein and it is used as an indicator of inflammation [5]. A possibility of change in the effect of cinnamon may occur with the utilization of cinnamon nanoparticle because the alteration in the physical and chemical characteristics such as melting point, conduction of electricity, magnetic permeability and alterations in chemical reactivity of a particular substance occurs with the reduction in the size of particle to the level of nanometer. These unique physiochemical properties may be attributed to the increase in the surface area which occurs due to the reduction in the size of particle [6]. With the increase in the surface area of a substance, there is a greater contact of that material with the surrounding substances which may influence the effectiveness of the reaction. The total surface area and 1x1 cm mass in the form of 1mm cubes is 60 cm2, while the total surface area of 1x1 cm mass in the form of 1nm cubes is 60,000,000 cm2. This significant increase in the surface plays a very vital role as far as the physiochemical properties of the material are concerned. The specific physical and chemical properties may also exert their influence on the interactions between the nanoparticles of a specific substance to a biological tissue [7]. The nanoparticles of a specific substance may enhance the beneficial biological effects but may also cause an increase in the toxicity. In this regard, it would be imperative to evaluate the toxic effects of the nanoparticles of the certain useful substances. The present study is aimed to evaluate the toxic effects of nanoparticles of cinnamon on the liver and kidney of the rats by histopathological examination.

Material and Methods

Synthesis of cinnamon nanoparticles

The cinnamon nanoparticles were obtained by method of reduction and selection of size. For this process, 10 g of micro particles of commercially available cinnamon powder were obtained. These micro-particles were crushed for 6 hours by utilizing a micro-mill vibratory consisting of a grinding set (mortar and agate ball) and passed through sieve Ro-tap model E test sieve shaker keeping the sample in constant motion for 15 min on a 20 μm mesh. Through this process, fine particles were filtered. This process was repeated 2 more times. These synthesized nanoparticles were subjected to various characterization techniques such as X-ray crystallography (XRD), Scanning electron microscope (SEM), and Energy-dispersive X-ray spectroscopy (EDS).

Group formation

After the approval of the ethical committee of the International Islamic University Islamabad, this experimental study was carried out.

The mice were divided into two groups (Group A and Group B). The Group A contained experimental animals and Group B contained six animals (BALB/c mice) as control. The Group A animals were further divided into three subgroups (A1, A2, and A3). Each subgroup (A1, A2 &A3) contains six BALB/c mice. The subgroup A1 rat animals were fed with cinnamon nanoparticle 5 ppm. The subgroup A2 mice were fed with cinnamon nanoparticle 10 ppm while the subgroup A3 mice were fed with cinnamon nanoparticle 20 ppm.

Histopathological examination of specimens

The liver and kidney samples of the control group (B) and experimental groups (A1, A2, and A3) were evaluated for the possible toxicological effects by the histopathological examination. The specimens were fixed in 10% buffered formalin and processed in an automated tissue processor. After completing the tissue processing, the tissue was embedded in paraffin wax and blocks were prepared. From these blocks, 3- 4 micron thick sections were cut using a microtome. The stained slides were evaluated by two pathologists independently.

Results

Characterization of synthesized cinnamon nanoparticles:

Being an organic molecule, the X-ray crystallography of these synthesized cinnamon particles showed several peaks which can be seen in Figure 1. Several peaks showed that the cinnamon particles constitute of several elements and is an organic molecule. Several peaks at the x-axis showed that there are many constituents with different particle sizes. The size of these particles was estimated by employing the Debye-Scherrer method. The scanning electron microscopy revealed that these synthesized particles are in nano scale. It was observed that these several different elements which constitute cinnamon were of different shapes and sizes. Figure 2 shows the scanning electron microscopic (SEM) image.

Energy-dispersive X-ray spectroscopy was conducted to evaluate the constituents of the synthesized cinnamon particles. From the observation of Figure 3, different elements can be seen which makes up this organic molecule.

Histopathological evaluation:

The sections from the liver tissue have been examined under a light microscope for evidence of balloon degeneration, necrosis, apoptosis, inflammation, and fibrosis. The sections from control group (B) and experimental groups A1 and A2 revealed no significant histopathological abnormality while the Group A3 exhibited grade 1 inflammation in the liver (Figure 4).

The sections from kidney tissue were evaluated for the histological evidence of any toxic effects on the glomeruli, tubules, interstitium and blood vessels. The sections from control group (B), experimental groups A1 and A2 revealed unremarkable glomeruli, tubules, interstitium and blood vessels. The section from group A3 showed vacuolization in the tubules while the glomeruli and blood vessels of this group revealed no significant histopathological abnormality (Figure 5).

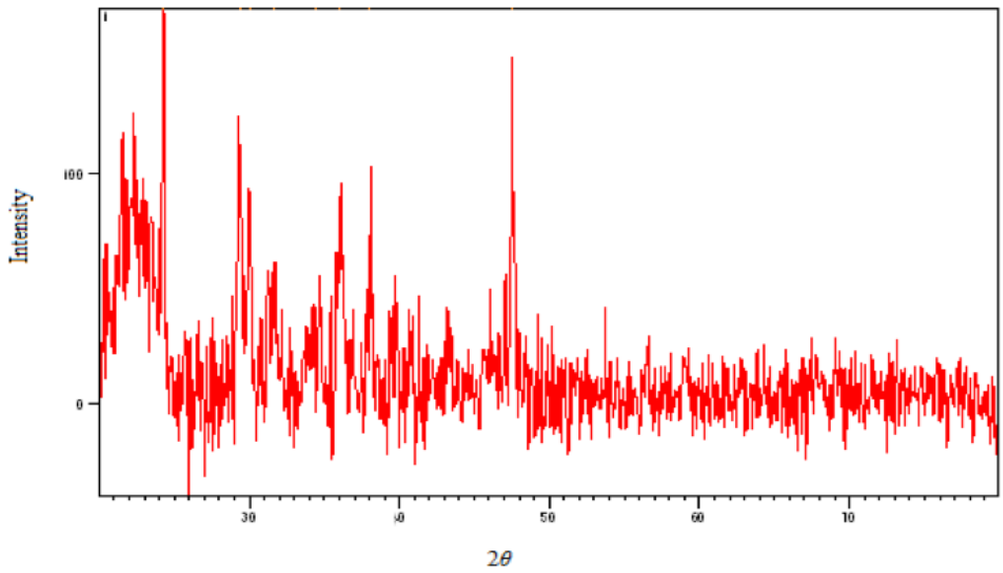

Figure 1. X-ray diffraction graph of cinnamon nanoparticles

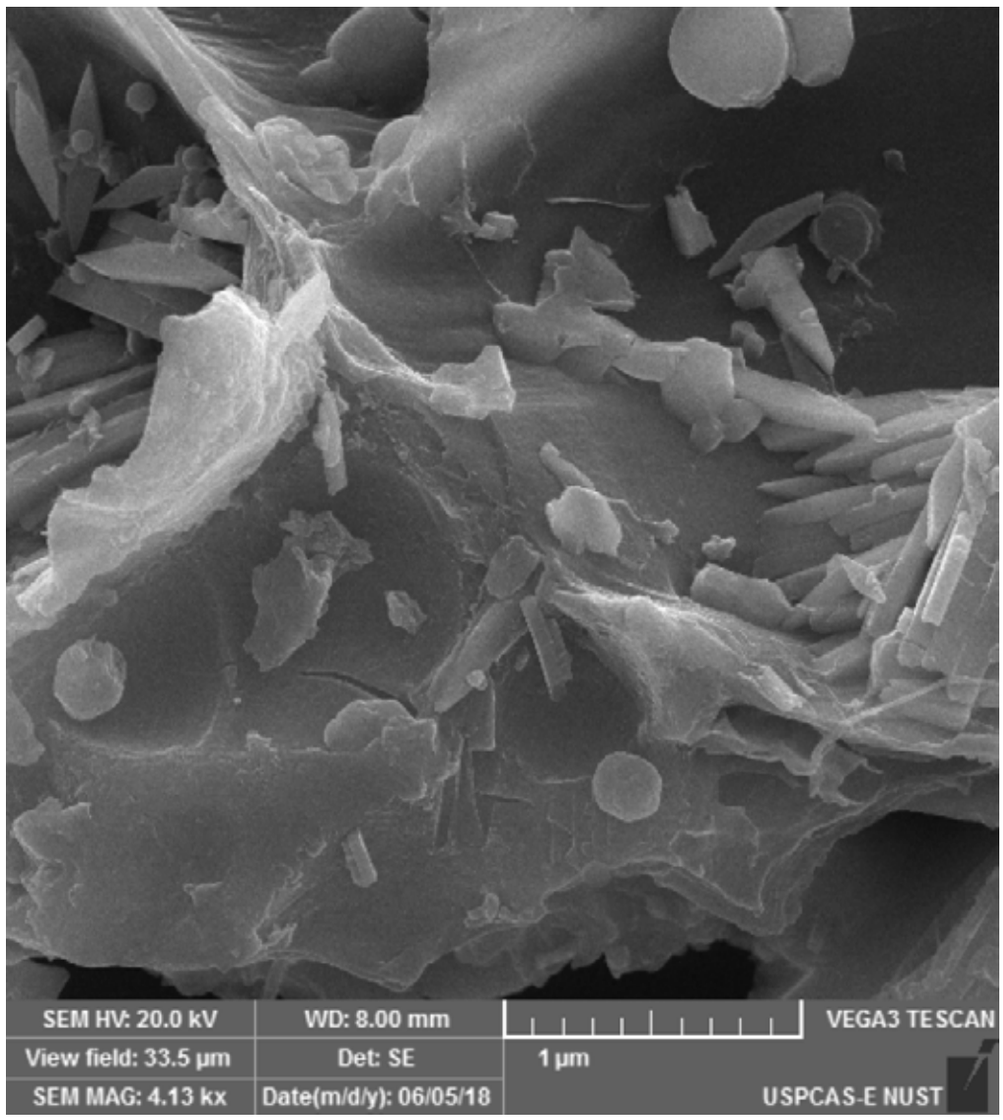

Figure 2. Scanning Electron Microscopic image of synthesized cinnamon nanoparticles

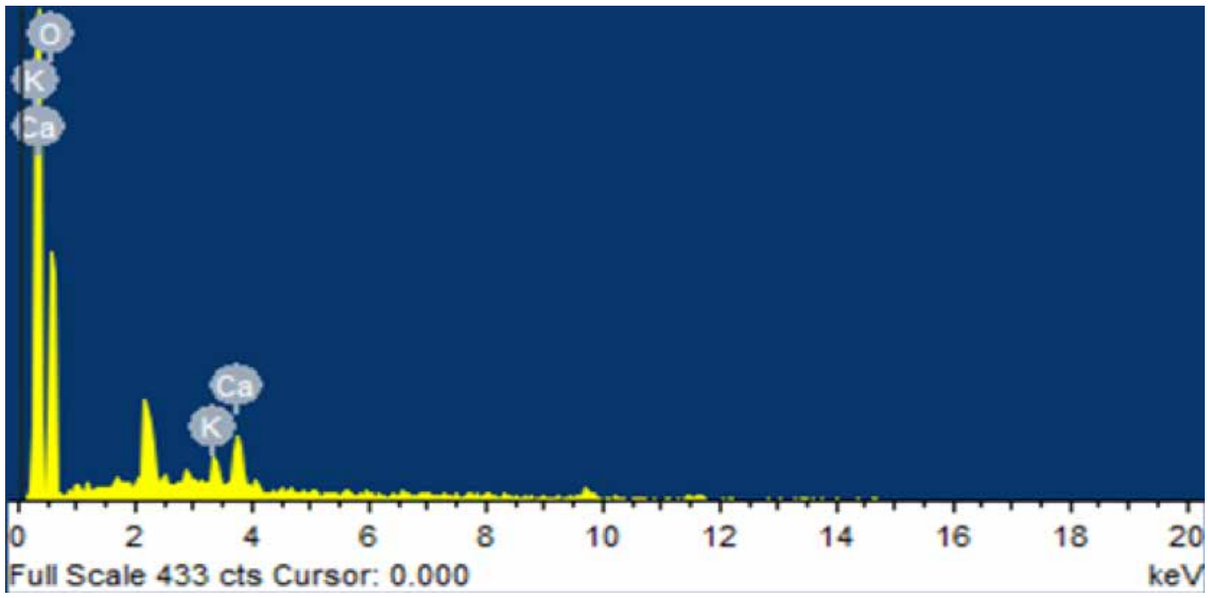

Figure 3. Energy dispersive X-ray spectroscopy pattern of synthesized cinnamon nanoparticles

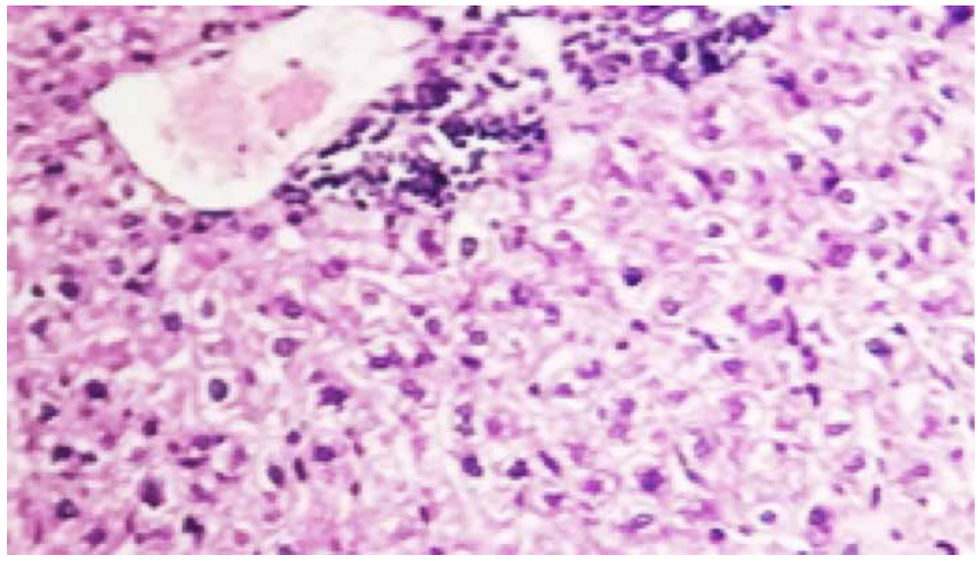

Figure 4. Histopathological section of Liver specimen (from Group A3) administered with high dose of cinnamon nanoparticles. White arrows point to the inflammatory cells (H&E x200)

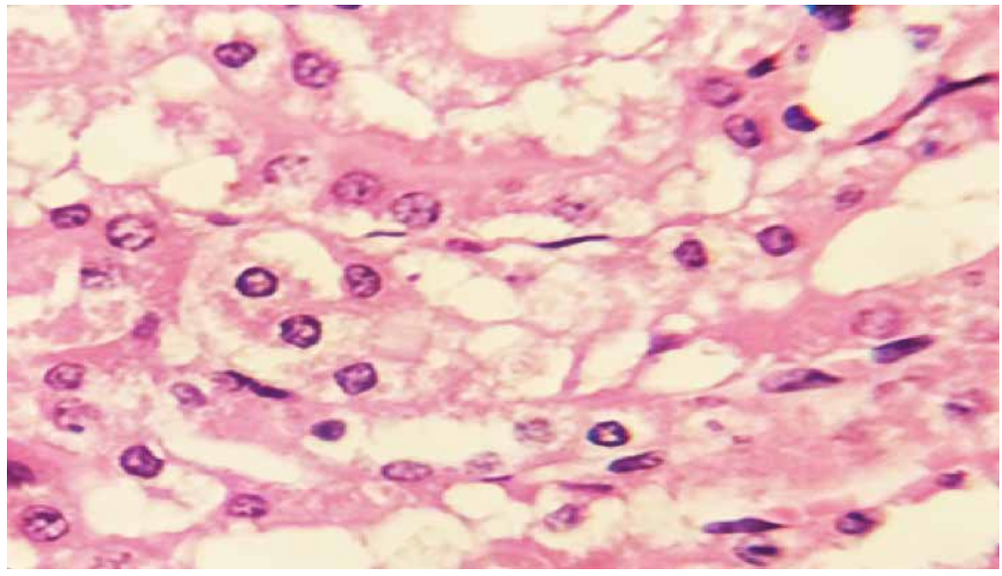

Figure 5. Histopathological slide of Kidney specimen (from Group A3) administered with cinnamon nanoparticles (H&E x400).

Discussion

Cinnamon is a quite commonly used substance all over the world. Apart from its delicious taste and flavouring of the food, it has been an effective remedy against many ailments since ancient times. The studies have revealed that the use of cinnamon helps in controlling blood glucose level and it has protective effects against oxidative stresses [8, 9]. Along with the beneficial effects of the cinnamon, there are no toxic and side effects associated with the use of cinnamon. A phase 1 clinical trial has been performed on the healthy adults for three months with the doses of 85 mg, 250 mg, and 500 mg of the water extract of Cinnamomum zeylanicum in the University of Colombo which revealed that there was no significant change in the blood cells counts, liver functions tests, and kidney function tests [2].

With the rapid emergence of Nano-science, nanoparticles of many substances are being synthesized and assessed for the possible beneficial properties in many fields such as chemistry, electronics, and medicine. One of the important areas in the field of medicine is to evaluate the effects of nanoparticles for the treatment of viral, bacterial, fungal, and parasitic infection with particular focus on the multidrug- resistant pathogens. In one study, the nanoparticle from cinnamon has been found effective as an antiviral agent against the H7N3 influenza A virus in the Vero cells without any toxic effects on these cells [10].

Another important area is the prevention of cancers and many other diseases which are associated with the increased production of free radicles. The evaluation of the nanoparticle for the prevention of oxidative stress has been of a primary focus.

An experimental study of nanoparticle from dietary cinnamon has been conducted on Nile tilapia fish which revealed that the activity of antioxidants like superoxide dismutase and catalase significantly increased as compared to control and similarly the secretions of lipase, amylase, and protease have been induced with the supplementation of cinnamon nanoparticles [11].

Most of the conducted studies are focused on the anti-microbial and anti-oxidative effects of nanoparticle and there is less focus on the untoward effects associated with the dose of nanoparticle. In the present study, the different dose strengths were given to different groups of mice for the assessment of possible toxic effects on the liver and kidneys. The current study revealed that no histological evidence of the toxic effects of cinnamon nanoparticles with the dosage of 5 ppm and 10 ppm has been observed in the liver and kidney of the mice but the dosage of 20 ppm revealed certain histological alteration in the liver and kidney of the mice.

A study published by Kouame K et al. narrated the toxic effects of long term use of cinnamomun cassia silver nanoparticle on the kidney and liver of Sprague - Dawley rats [12]. In their study, they detected that the rat exposed to 10 mg/kg of silver nanoparticles synthesized from the cinnamomum cassia extract revealed pyknosis, vacuolar changes and distortion in the arrangement of hepatocytes of liver specimen of rats. The degenerative changes were more severe as compared to 5 mg/kg of silver nanoparticles (from cinnamomum cassia extract). In the kidney specimen, they found mild morphological alterations in the glomeruli of the rat kidney which were given 5 mg/kg of silver nanoparticles (from cinnamomum cassia extract) while changes were more severe and generalized in the kidneys of rat exposed to 10 mg/kg of silver nanoparticles. It suggests that the increasing dose of nanoparticles produces more damaging effect [12]. In our study, the morphological changes were less severe than the study published by Kouame K et al. but these are similar in this aspect that in both series, increase in the dose of nanoparticles increases the impact of damage. A study published by Yun JW et al. showed that the use of a high dose (2000mg/kg) of cinnamon extract is associated with hepatotoxicity and nephrotoxicity [13].

The application of nanoparticles is emerging as a potential tool for the diagnostic and therapeutic purposes in the field of medicine. It would be appropriate to extensively study the potential health hazards of these nanoparticles with respect to their size and dose. Further studies with different sized nanoparticles and their variable doses may be of paramount importance in the enrichment of knowledge regarding these potential therapeutic medicines and diagnostic tools for the effective and safe patient care.

Conclusion

Nanoparticles of cinnamon show some histological evidence of toxic effects on the liver and kidney specimens of rats with a higher dosage.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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