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Original Research Article

EVALUATION THE HEPATOPROTECTIVE EFFECT OF QUERCETIN AGAINST ZINC OXIDE NANOPARTICLES INDUCED TOXICITY IN MOUSE MODEL

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Abstract: *Quercetin is a well-known flavonoid that resents in fruits and vegetables. Zinc oxide nanoparticles (ZnO-NPs) are widely used for packing and cosmetics. This study was conducted to determine the effect of Quercetin on the liver by ZnO-NPs toxicity in a mouse model. Thirty-six Swiss albino mice were divided into two groups, i.e. control group and the experimental group. The control group was administered saline for 28 days orally. The experimental group is further divided into three groups. To the first one, only ZnO-NPs (30 mg/kg, orally) were given for 28 days. To the second group, only quercetin (100 mg/kg, orally) was given for 28 consecutive days. To the third, a combination of quercetin and ZnO-NPs was given for alternate 28 days through the oral route. Animals were sacrificed after 14 and 28 days of dosing. Mouse liver was fixed in the 10% formalin to preserve organ integrity. Histopathological analysis was done to observe the structure of liver in treated mice. The ZnO-NPs group shows the central vein full of infiltrations, sinusoidal spaces congestions and some the necrosis of hepatocytes was observed. The quercetin group shows a normal central vein with no infiltrations. The animals that received both quercetin and ZnO-NPs showed infiltration in central vein and necrosis but less than that of only ZnO-NPs given group. Full recovery in the liver structure was observed in the quercetin+ZnO-NPs group after 28 days of treatment. This study showed the hepatoprotective effect of quercetin because it reduces the toxicity produced by ZnO-NPs.*

Keywords: Quercetin, zinc oxide, nanoparticles, liver, mouse model

Novelty Statement

This study reports that the quercetin has very strong hepatoprotective and anti-inflammatory properties.

Introduction

Nanoparticles (NPs) use has increased drastically due to its use in modern technologies, but its harm cannot be evaluated on human health due to insufficient data. NPs started to attract attention due to their chemistry, small size, non-biodegradability and reactive surfaces. They can easily disperse in the environment without any consequences (Wang *et al.*, 2009).

NPs cause adverse human health issues, depending upon their nature, size, shape and origin of toxicity. Metallic NPs can generate reactive oxygen species (ROS) and reactive nitrogen species (RNS) due to redox-cycling reactions. Previous research have shown the toxicity of NPs depending on the reactive oxygen species they generate (Xia *et al.*, 2006; Zhang *et al.*, 2010). Metallic NPs cause oxidative stress in the normal cellular process in the body that can be harmful if the oxidative stress increases. Recent studies have evaluated the toxicity of ultrafine nanoparticles concerning the oxidative stress cause by the metallic NPs (Huang *et al.*, 2010).

Nanotechnology use has increased worldwide due to its application in diagnosis, drug delivery systems, food industry, paints, cosmetics, electronics and sports (Schilling *et al.*, 2010). NPs Toxicity

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mechanism is unknown, but depending upon its ability to change phytochemical and structural properties, it may be responsible for causing the toxicological effect in the human body. These changes may be surface-specific groups (carbonyl group, thiol groups) or the presence of reactive sites (Hussain *et al.,* 2009). The reactive sites can either act as electron acceptors or electron donors depending upon the nature of NPs. It generated superoxide radicals of oxygen which can generate reactive oxygen species by Fenton reaction (Cabiscol *et al*., 2000).

Many NPs are used in engineering nanotechnology, but zinc oxide nanoparticles (ZnO-NPs) are one of the most widely used products in cosmetics and paints. It is because ZnO-NPs can absorb UV without scattering the visible light and efficiently absorb infrared radiation. ZnO-NPs FSNB microbial agent in the Fort industry for additives and packing. They can also be used as a fungicide in agriculture and anti-cancerous drugs and for the imaging of biomedical applications (Gerloff *et al.,* 2009). In mammalian cells, ZnO-NPs cause membrane injury, inflammation, DNA damage and apoptosis (Rasmussen *et al.*, 2010).

Quercetin is an active phenolic compound present in over 4000 plants. It was first identified 1936 by Szent-Gyorgyi in 1936. It is an important flavonoid, considered an antioxidant and must be consumed regularly in diet. It was initially amed as vitamin P and later named as quercetin. Quercetin ($C_{15}H_{10}O_7$) is a yellowish crystalline powder with a molecular mass of 302.236 g/mol and a density of 1.8g/cm^3 (Ames *et al.,* 1975). Quercetin acts as hepatoprotactant and lessens liver fibrosis against many diseases (Bengmark *et al.,* 2009).

Materials and methods

Chemicals and reagents

ZnO-NPs were purchased from U.S Research Nanomaterials, Inc., while the quercetin was purchased from General Nutrition Corporation Pittsburgh, PA-15222.

Animal selection and grouping

Thirty-six healthy Swiss albino mice of varying ages, between a few weeks to one-month-old, weighing 30-45 gm were purchased from local market. All mice were kept in steel boxes, and a 12-hours light and dark cycle was maintained, and temperature in the experimental room was kept at 25^{o}C. To maintain temperature, electric heaters were used. Animals were allowed to acclimate to laboratory conditions for a week and were kept in the Government College University, Lahore animal house facility.

Animals were fed on commercial rodent chow in pellet form, which contains at least 16% protein and 18% fiber, not more than 4% fat. Drinking water was provided throughout the experiment.

Thirty-six mice were randomly divided into four groups of 9 animals each and were treated as follows for 28 days, orally:

Group I: (n=9) 0.9 % saline was given for 28 days consecutively.

Group II: (n=9) Quercetin (100mg/kg, b.w.) was given to mice for 28 consecutive days.

Group III: (n=9) 0.9% saline was given for 7 days, followed by ZnO-NPs (30mg/kg, b.w.) for 21 alternate days.

Group IV: (n=9) quercetin (100mg/kg) was given for 7 days, followed by both ZnO-NPs and quercetin in combination for the next 21 days.

Organ procurement:

Animals were anaesthetized with ketamine (10mg/kg), and injected intraperitoneally. Organ was washed in ice cold saline and stored in 10% formalin for the histological examination.

Histopathological Investigation:

The liver samples were previously fixed in 10% paraformaldehyde. The excess water from the tissues was removed by placing the tissue in different grades of alcohol (80%, 90% and 100%) for half an hour in each grade. The tissue was then placed in cedar wood oil and allowed to stand in paraffin for 12 hours, so the tissue was completely infiltrated by paraffin. Then the tissue section was embedded in paraffin, and blocks were made. Tissues were sectioned by microtome into 4-5 μm thick sections. These sections were fixed into slides, stained with haematoxylin and eosin (H/E), and mounted by DPX for microscopic observations (Mark *et al.,* 2013).

Results:

Liver cells were unchanged in the control group

The histology of control group animals throughout the study showed the same results. It revealed the defined structure of the central vein of the liver, having a regular sheet of hepatocytes and well-defined portal vein. It is free of infiltration of red blood cells. Sinusoidal spaces were visible between the sheets of hepatocytes. Regular radial hepatic cords emerge from the central vein towards the margins form the hepatic lamina (Figure 1).

Effect of quercetin on liver cells

No histopathological alterations are observed in the liver of albino mice after treating quercetin (Figure 2).

Effect of ZnO-NPs on liver cells

There was a distortion of the central vein, some the infiltration in the portal vein, sheets of hepatocytes are irregular and hepatic lobule is distorted (Figure 3) after the treatment of ZnO-NPs. These changes increased after 28 days of treatment of ZnO-NPs in albino mice live.

Effect of quercetin + ZnO-NPs on liver cells

When combinations of quercetin and ZnO-NPs were given to mice, it first showed swelled hepatocytes, congested sinusoids and infiltration in the central

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vein. But after 28 days of treatment, the liver structure is becoming again like the control group but with few infiltrations, which shows the hepatoprotective role of quercetin on the liver of albino mice when ZnO-NPs induced the toxicity.

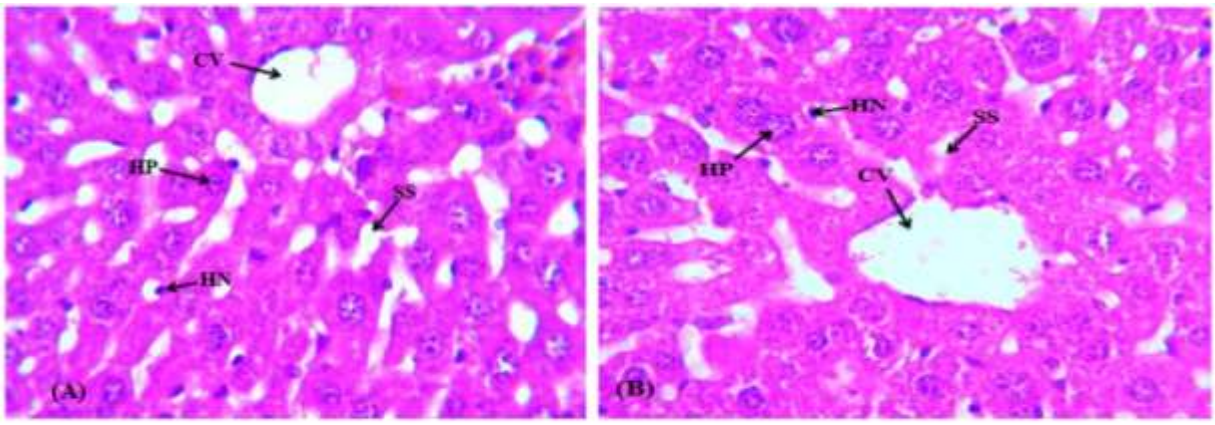

Figure 1: Cross section of liver of control group of **(A)** 14 days **(B)** 28 days. Central vein (CV), hepatocytes (HP), sinusoidal spaces (SS) and hepatic nuclei (HN). (H & E)

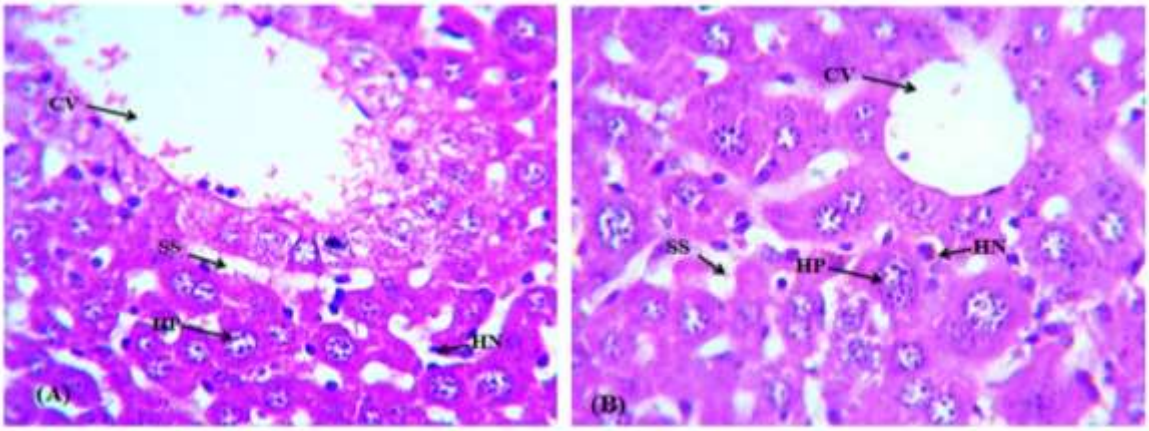

Figure 2: Cross section of liver of quercetin treated group of **(A)** 14 days **(B)** 28 days. Central vein (CV), hepatocytes (HP), sinusoidal spaces (SS) and hepatic nuclei (HN). (H & E)

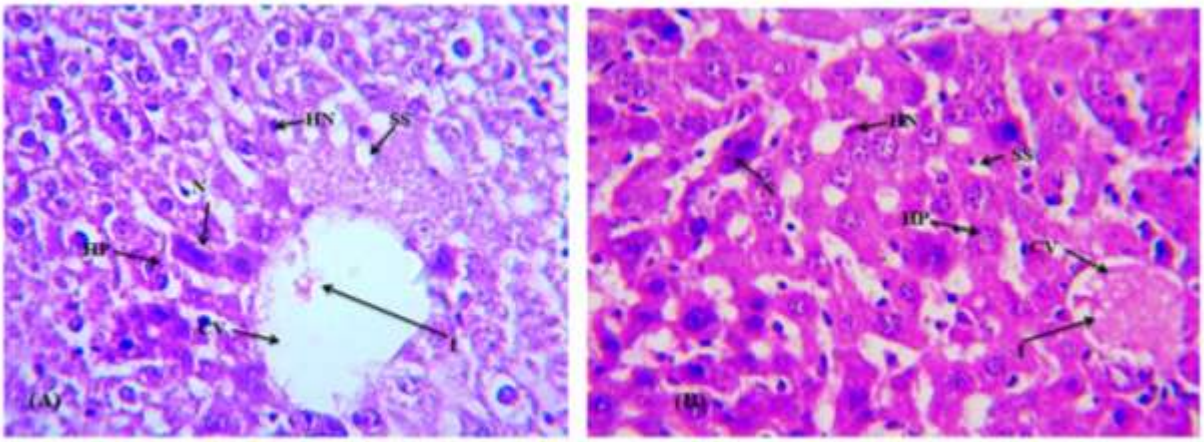

Figure 3: Cross section of liver of ZnO-NP treated group of **(A)** 14 days **(B)** 28 days. Central vein (CV), hepatocytes (HP), sinusoidal spaces (SS), hepatic nuclei (HN), necrosis (N) and infiltration (I). (H & E)

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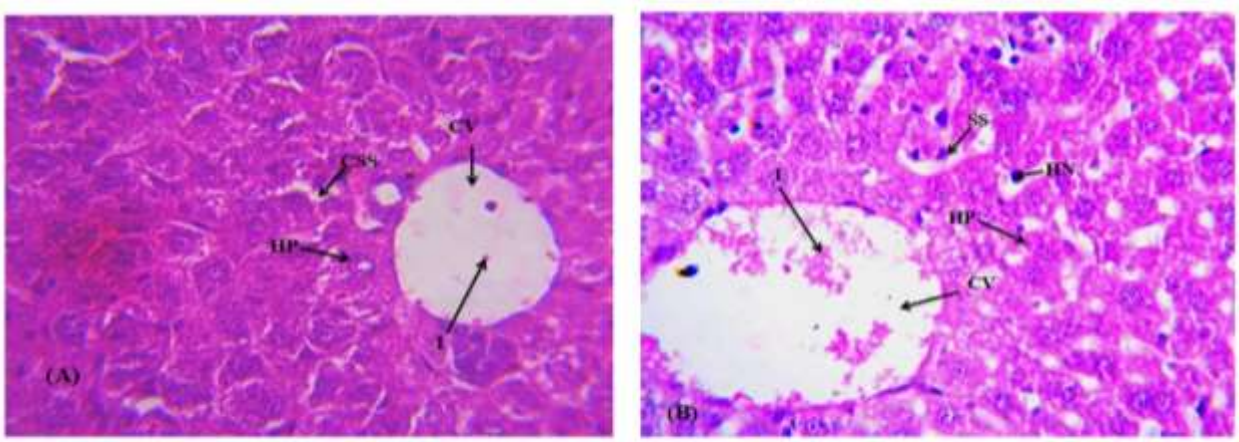

Figure 4: Cross section of liver of quercetin + ZnO-NP treated group of **(A)** 14 days **(B)** 28 days. Central vein (CV), hepatocytes(HP), congested sinusoidal spaces(CSS), hepatic nuclei(HN) and infiltration(I). (H & E)

Discussion

The complexity of liver problems nowadays seems to have increased. We may come into contact with chemicals and other environmental pollutants frequently, contributing to some of this complexity. There has been a significant rise in the amount of medication or other hazardous chemicals eaten, which could harm the liver (Amin and Hamza, 2005). Antioxidants were given to animals to study their effect. The route of administration was either intraperitoneal or oral, depending on the absorption or availability of antioxidants. Liver plays an important role in the metabolism of foreign particles and minimizes the oxidative stress caused by them. It may cause different diseases in the body, such as diabetes and kidney failure. So, animal models have to be modified to improve the antioxidant effect. Due to the lack of animal models, antioxidants show desirable effects in them but did not reproduce same effects in human clinical trials which acts as a barrier to developing the effective treatment of the concerned disease. So, to develop the antioxidant therapy, translational research is one of the best tools to consider. It helps to select the dose and duration of antioxidants treatment in the presence of ROS in different situations and between animals and humans (Li *et al.,* 2015).

The present study shows that, after giving the quercetin for consecutive 28 days, there is no significant change in the live structure. There is also the same structure as that of control group but some protective effects can occur. There is normal central vein, sinusoidal spaces and hepatocytes and no significant increased liver size. Quercetin, an antioxidant, has exhibited beneficial effects by increasing the enzymatic activity and pro-oxidant effect against liver stress in mouse. Quercetin can potentially scavenge reactive species such as superoxides and peroxyl radicals. Administrating the Quercetin also (Choi *et al.,* 2003).

When oxidative stress is applied to the body it can cause liver fibrosis mainly by increasing the activation of stellate cells and the collagen synthesis. When hepatotoxins are applied, they can cause damage to perivenular hepatocytes as they contain less antioxidant defence. (Yoshio *et al.,* 1987). Our results showed that Zno-NPs caused hepatocellular changes in the liver as it retained more esinophilic stain and less amonophilic compared to cytoplasmic ratio. Drak brown staining positively suggested the presence of iron in Kupffer cells. It was concluded that ZnO-NPs caused alterations in the liver by causing necrosis of hepatocytes and increased infiltration in the central vein.

Nanoparticles affect the human body depending upon its size, shape and agglutination in the body. Also, the human genetics and environmental factors play an important role in causing the damge to tissues and cells (Buzea *et al.,* 2007). When the toxicity causes damage to the liver, it, in return releases the enzymes in the body, which causes oxidative stress to nearby tissues. The role of oxidative stress in the mechanism of NPs induced hepatotoxicity has also been reported by Sha *et al,.* 2014. The current study aims to investigate the sub-acute toxicity of ZnO-NPs mechanism in mouse. For this purpose, oral route of administration was selected as many food packaging contained nanoparticles which can be transferred to humans through the gastrointestinal tract. Nanoparticles are also present in cosmetics asmajor sources of ingestion during its use. Considering all these factors, we selected the sub-acute dose of ZnO-NPs based on the literature for in vivo study of an animal model. Metal oxide nanoparticles administration at a safe dose did not show any mortality, as was confirmed by previous researchers Sharma *et al.,* 2012 and Sycheva *et al.,* 2011.

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Nanoparticles treatment caused the swelling of hepatocytes. It dilates the central vein and also penetrates the blood into sinusoidal spaces. It can be concluded that nanoparticles decrease the cell membrane permeability of hepatocytes and blood vessels endothelial lining. This liver damage can cause the deposition of fats by reducing the fat metabolism and increasing lipid peroxidation. This led to lipolysis of hepatocytes by ZnO-NPs which further caused liver damage (Ma *et al.,* 2010). Necrosis and fat deposition can be seen in liver histology. The ZnO-NPs were given through the gastrointestinal tract, which in turn caused the damge to live. It means that ZnO-NPs are transported from one part to another in the body (Liu *et al*., 2009; Ma *et al*., 2009).

Quercetin ameliorates the altered enzyme level and protects the mice's liver against chemicals and drugs induced hepatotoxicity. The hepatoprotective effect of quercetin on liver is well evident, which significantly inhibits the elevation of enzymes in rats by keeping the structural integrity of liver. The hepatoprotective effect of Quercetin in the present study for 4 weeks showed that it ameliorates liver injury in mice intoxicated by ZnO-NPs, as zinc oxide, a well-known model compound for induction of hepatic injury.

Conclusion

The present study concluded that quercetin has anti-inflammatory and anti-cancerous properties, acts as hepatoprotectant and affects the liver cells and prevents cell necrosis and fibrosis. Quercetin also prevents the damage caused by the oxidative stress of zinc oxide. Quercetin does not affect the weight gain and loss of mice but prevents inflammation. Histological study also showed liver recovery after quercetin administration with induced zinc oxide nanoparticle toxicity demonstrating the protective and regenerative effect. Quercetin helps maintain the central vein, hepatocytes and sinusoidal spaces. It shows some of the infiltration of red blood cells in the portal and central veins due to external stresses. When ZnO-NPs induce the toxicity, the liver shows the dilation of the central vein, hepatocytes become swelled and the congestion occurred in sinusoids. As compared to control group, which shows normal central vein, hepatocytes, sinusoids and kupffer cells. When both quercetin and ZnO-NPs were given to the same mice, the structure of the cells changed. The central vein in the 14 days gets dilated as compared 28 days of treatment. The sinusoidal spaces get congested first, and then the recoveries of cells are observed. So, the hepatotoxicity caused by ZnO-NPs can be reduced by giving the quercetin to the same animal. The quercetin helps maintain the normal functioning and the cell's structure. Results demonstrate the positive effect on liver functions.

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Conflict of interest

The authors have declared no conflict of interest.

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