INTRODUCTION

Recently, scientific research on NPs has shown a great and rapid growth due to their wide applications in various fields, including electronics, cosmetics, food industry, and medicine. The use of NPs has great potential in a wide range of applications, since their distinct physicochemical characteristics can be utilized in an application-specific manner by modifying their size, surface properties, and shape. Therefore, there is increasing probability for human and environmental contact with these nanomaterials ***(Zhou et al., 2016)***

However, their unique physicochemical properties may affect their toxicological behavior in vivo by facilitating cellular uptake and translocation of the particles in the body. Furthermore, the physiological medium influences the interaction between biological systems and NPs and can determine the fate and biosafety of NPs ***(Lee et al., 2016)***.

Metal oxide nanoparticles, such as CuO, have attracted attention mostly because of their antimicrobial and biocide properties. They are used for the development of many dental and surgical instruments, such as dental composite and intra uterine contraceptive devices ***(Devi et al., 2014).***

In addition; CuO is a semiconductor metal with unique optical, electrical and magnetic properties and it has been used for various applications, such as the development of supercapacitors, near-infrared filters, in magnetic storage media, sensors, catalysis and semiconductors ***(Grigore et al., 2016)***

Although CuO NPs have proved their use in biomedical applications; the major disadvantage for their use on the medical field is due to their potentially toxic effects. The main toxicity process relies on the increased production of reactive oxygen species.These nanoparticles induce oxidative stress in human cells, promote toxicity and can damage DNA and mitochondria ***(Ruiz et al; 2015 & Bugata et al., 2019).***

It is reported that kidney, liver and spleen are the target organs of CuO NPs ***(Gosens et al., 2016)***.

Spleen is the largest secondary lymphoid organ with a complex vascular and cellular organization. The spleen induce immune response against blood antigens, and defend against invading bacteria, fungi, viruses and other infective agents. The spleen represents an important clearance site for nanoparticles

due to its high blood flow and loose capillaries ***(Cataldi et al., 2017).***

Exogenous antioxidants like vitamin C, vitamin E, and beta carotene or vitamin A are among the most widely studied dietary antioxidants. Vitamin C is considered the most important water-soluble antioxidant in extracellular fluids ***(Nimse et al; 2015 &Jafari et al., 2019)***.

AIM OF THE WORK

The aim of this study was to investigate the effect of different doses of copper oxide nanoparticles on the histological structure of the spleen of adult male albino rats and to assess the possible protective role of vitamin C as an antioxidant.

HISTOLOGICAL STRUCTURE OF THE SPLEEN

The spleen is the largest lymphatic organ. It is located in the upper left quadrant of the abdominal cavity and has a rich blood supply ***(Alex et al., 2015).***

The spleen is the only lymphatic organ involved in filtration of blood, making it an important organ in defense against blood-born antigens. Any inert particles in the blood are actively phagocytosed by spleen macrophages ***(Ross et al., 2016)***.

The spleen is formed of stroma and parenchyma.

- ***The stroma:***

The spleen is surrounded by a **capsule** of connective tissue, elastic and smooth muscle fibers from which emerge **trabeculae** which partially subdivide the parenchyma or splenic pulp.

- ***The parenchyma:***

The spleen pulp has two components; the red pulp & the white pulp.

The red pulp consists of blood filled venous sinusoids and splenic cords of Billroth, while the white pulp consists of lymphoid nodules and periarteriolar lymphatic sheathes (PALS) ***(Alex et al., 2015).***

- ***The red pulp:***

Red Pulp constitutes about 70 % of the total splenic volume in adult. Red pulp contains numerous sinuses which are filled with blood rich in platelets. Elongated endothelial cells line the sinusoids of the spleen with their long axes parallel to the long axes of the sinusoids. These cells are enveloped in reticular fibers set primarily in a transverse direction, much like the hoops on a barrel ***(Parham et al., 2014)***.

Surrounding the sinusoid is an incomplete basal lamina. Because the spaces between the endothelial cells of the splenic sinusoids are 2–3 μm in diameter or smaller, only flexible cells are able to pass easily from the red pulp cords to the lumen of the sinusoids ***(Treuting et al., 2017).***

The sinuses are lined by unusual, non squamous cells with large bulging nuclei. They are called stave cells and have special properties that allow selection of healthy red blood cells in splenic cords ***(Alex et al., 2015)***.

Between the sinuses, spongy cellular cords (cords of Billroth) are found. Cords are made up of reticular fibers and reticular cells intermingled with a number of immune cells, such as macrophages, dendritic cells, monocytes, granulocytes, B cells, T cells and plasma cells. Red pulp plays an important role in blood filtration, antigenic stimulation and proliferation of B and T cells and production of antibodies of different specifications ***(Treuting et al., 2017)***.

- ***The white pulp: PALS and Lymphoid Follicles:***

The white pulp (WP) is a lymphocyte rich area which contains **PALS** around the arterial vessels particularly around the central arterioles and **lymphoid follicles** ***(Parham et al., 2014)***.

The PALS is a sheath of lymphocytes mostly $CD4^+$ T cells that envelope the central arterial vessels. These $CD4^+$ T cells are defined as helper T cells within the immune system ***(Alim et al., 2012)***.

Regarding the lymphoid follicle it does not only contain B cells but also T cells, which are found adjacent to the PALS in addition to some macrophages and follicular dendritic cells. Significant immunological activities, cell

trafficking and cross talk between various immune cells occur in the follicles ***(Alim et al., 2012)***.

When lymphoid nodules become activated as a result of the arrival of antigens, the lymphocytes proliferate in the central portion of the nodule which then stain lighter and is called a germinal center ***(Alex et al., 2015)***.

The marginal zone is the region at the interface between the red pulp and the white pulp of the spleen consisting of many blood sinses and lymphoid tissue. It is a highly transited area that receives large amounts of blood from the general circulation. It is the site where antigens in blood come in contact with parenchyma of the spleen. In addition to the marginal zone B-cells that normally reside there, a number of other cell types that are present in the blood pass through the marginal zone e.g. lymphocytes and granulocytes. In addition, a large number of dendritic cells are thought to reside temporarily in the marginal zone before migrating into the white pulp following stimulation and antigen uptake. Moreover, a large number of lymphocytes remain in the marginal zone for a period of time during the process of transmigration into the white pulp ***(Torres et al., 2014).***

Following antigenic stimulation, positive selection and clonal expansion of B cells occurs in both the marginal zone and the germinal center of the lymphoid follicle**.** The opening of the arterial blood stream in the marginal sinuses results in a reduction of the velocity of the blood stream, so antigens are initially screened in the marginal zone. Therefore, potent phagocytic cells; the marginal zone macrophages; are present which can take up and phagocytose large foreign particles, such as bacteria and defective red blood cells. Marginal sinuses are continuous with vessels that feed the capillary beds of the PALS and follicles. These sinuses are lined by endothelial cells ***(Poluektova et al., 2017).***

- ***Blood supply of the spleen:***

The splenic artery divides into trabecular arteries located within the trabeculae entering the splenic parenchyma. The trabecular artery gives off central artery. The central artery branches out as central arterioles. These arterioles provide blood to the white pulp ***(Alex et al., 2015).***

Central arterioles are surrounded by sheaths of lymphoid tissues (PALS) at different points. Some of these terminate in the marginal sinus in the marginal zone, few extend beyond the white pulp to terminate in the red pulp. As

the central arterioles continue, the white pulp disappear and they become the penicillar arteries surrounded by red pulp. Blood from the red pulp collects in the venous sinuses which enter the trabeculae and merge into the trabecular veins. The trabecular veins do not have individual muscle walls. They can be considered channels hollowed out in the trabecular connective tissue and lined by endothelium. The trabecular veins then converge at the hilus to form the splenic vein which drains into the hepatic portal system ***(Ross et al., 2016)***.

- ***Differences between rat and human spleen:***

Histology of rat and human spleen is almost the same except for few differences. Megakaryocytes can be found in normal adult rat spleen in contrast to human spleen. In human spleen; megakaryocytes can be found only during embryo stage in which hematopoiesis occur in bone marrow, liver and spleen ***(Yang et al., 2013 & Lee et al., 2016).*** Later on, ***Mazen et al. (2017)*** confirmed that the adult rat spleen acts as a haemopoietic organ.

NANOTECHNOLOGY

- ***Historical perspective of nanotechnology:***

The history of nanomaterials reaches back thousands of years. Archaeologists found that 3000 years ago gold, silver, platinum and palladium nanoparticles were used in Egypt and China in paint, even without the knowledge of the existence and the properties of these materials. Since the medieval time, colloidal gold and silver were used in European churches for coloring glass as well as in the glaze of porcelain and other pottery ***(Horikoshi et al., 2013).***

Uropean Union defined NPs as natural or manufactured material, present in dispersed or aggregate state and their size usually under 100nm in diameter ***(Magaye et al., 2012).***

Before the last century, no analytical method existed to study materials of a size smaller than 1 μm, therefore the knowledge about those materials was limited ***(Horikoshi et al., 2013).***

Nanotechnology is a science that emerged in the beginning of the last century by the development of the high resolution microscopy techniques e.g transmission electron

microscopy (1932) and scanning electron microscopy (1937) ***(Heilitag et al., 2013).***

Types of nanoparticles:

Based on the sources used, there are mainly four types of nanoparticles as mentioned by ***Vance et al. (2015)*** and they are as follow:

- Metal oxide nanoparticles
- Metallic nanoparticles
- Polymeric nanoparticles
- Carbon based nanoparticles

Metal oxide nanoparticles:

Currently, interest has especially increased in metal oxide nanoparticles. These particles are widely used as industrial catalysts, chemical sensing devices, in medical applications, disinfection as antimicrobials, fillers, opacifiers and semiconductors. In addition, they are also useful in the field of cosmetics and microelectronics ***(Ahamed et al., 2014).***

These metal oxide nanoparticles exhibit unique chemical and physical properties. There are several types of

metal oxide nanoparticles such as Copper oxide (CuO), zinc oxide (ZnO), titanium oxide (TiO) and magnesium oxide (MgO).

Among various metal oxide NPs, CuO NPs have attracted particular attention because it is the simplest member of the family of copper compounds. Moreover, CuO NPs show a range of useful physical properties such as high temperature superconductivity, electron correlation effects, and spin dynamics ***(Frohlich et al., 2014).***

To investigate the toxicity of metal oxide NPs, several test systems and model organisms can be used. Many studies on the toxicity of different nanoparticles were carried out *in vitro* on cultured lung cells ***(Ivask et al., 2015).***

However, *in vitro* cell studies have their limitations and cannot mimic the entire organism. Therefore, to assess the whole picture of nanoparticle toxicity also *in vivo* inhalation and injection studies are necessary. Considering many comparative *in vitro* and *in vivo* studies on the potential toxicity of nanoparticles, it was shown that CuO NPs are among the most toxic metal oxide nanoparticles ***(Katsnelson et al., 2015)***.

COPPER OXIDE NANOPARTICLES (CUO NPS)

- ***Structure of CuONPs:***

Copper oxide (CuO) is a compound from two elements copper and oxygen. It is a crystal in which copper ion is coordinated by four oxygen ions ***(Singh et al., 2016).***

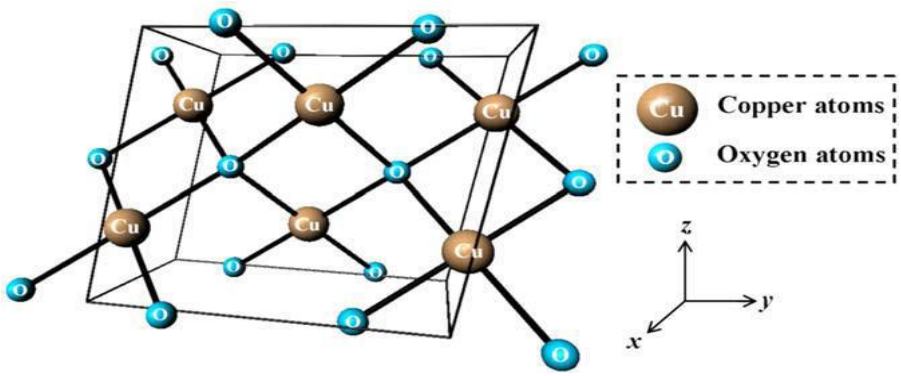

Fig. (I): Schematic representation of CuO Compound ***(Singh et al., 2016)***.

Applications of CuO NPs

- ***Biomedical applications:***

Copper oxide nanoparticles have attracted attention mostly because of their antimicrobial and biocide properties. In addition, they may be used in many biomedical applications. It is the most predominant application of CuO

NPs as it plays a central role in antimicrobial agents, disease diagnosis, drug design and delivery and wound healing ***(Nations et al., 2015)***.

1. Antimicrobial agents:

They are used in hospitals due to their antimicrobial ability to kill more than 99.9% of Gram-positive and negative bacteria within two hours of exposure, if a suitable dose is applied. Studies reported that the utilization of CuO reduces the occurrence of hospital-acquired infections. In addition, bed sheets containing CuO NPs are considered one of the most interesting innovations in medical care, since they reduce microbial attachment and thus microbial infections within hospitals ***(Lazary et al., 2014).***

Moreover, previous work had demonstrated that CuO NPs also have beneficial effects on the skin. Women who utilized pillow cases and beddings containing CuO NPs revealed an improved aspect of the facial skin. Additionally, due to its potent biocidal properties and being an essential trace element vital for the normal function of skin, CuO NPs impregnated socks has the capacity to kill fungi that causes tinea pedis (athlete's foot) and has the capacity to enhance skin elasticity as well ***(Dykes, 2014)***.