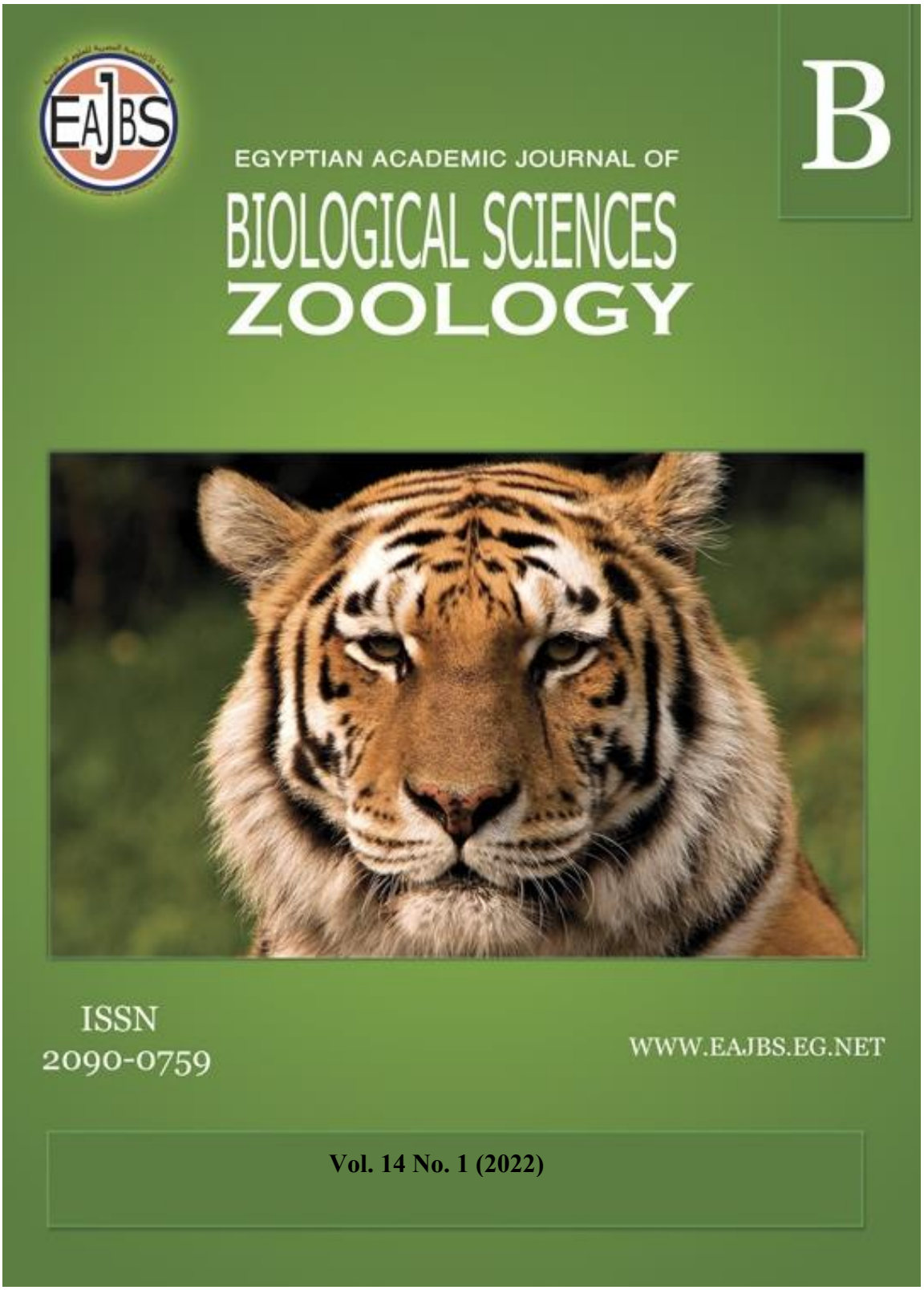

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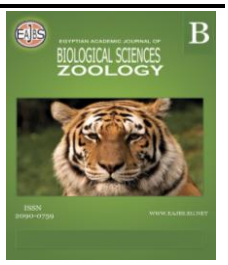

Protective Effect of Honey Against Zinc Oxide Nanoparticles' Toxicity in Maternal and Foetal Livers of Rat

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ABSTRACT

Zinc oxide nanoparticles (ZnO-NPs) used in many industries such as the food industry as additives and are a group of high crystallinity-nano particles. It is a toxic substance, especially when inhaled due to Zn2+ ion that is probably the main toxic material in vivo.

The present work was planned to study the protective effect of honey on pregnant rats' livers which received ZnO-NPs and their fetal livers. The pregnant rats were divided into six groups each was 6:8 pregnant rats. The first group served as a control group and received the distilled water. The 2nd group was administered 10 ml/kg per day of honey from the 6th to 15th day of gestation. The 3rd and 4th groups were administrated two doses of ZnONPs (10 and 50 mg/kg/day) respectively from the 6th to 15th day of gestation. The 5th and 6th groups received 10 ml/kg per day of honey before administration of ZnONPs (10 and 50 mg/kg/day). Animals of all groups received the administrated materials orally then they were sacrificed on the 20th day of gestation. The obtained results showed an increase in the pathological changes in the liver and an increase in the changes in the liver function with the two doses of ZnONPs as compared to the normal control group. Using natural honey reduces the histopathological changes caused by ZnONPs administration.

INTRODUCTION

Honey is a natural product that has been widely accepted as food and medicine by ancient and modern generations, civilizations and traditions (Israili, 2014). Honey is a good source of pharmacological or biological compounds that have antibacterial, antioxidant, anti-inflammatory, and antihypertensive activities (Alvarez-Suarez *et al*., 2014). The ameliorative effect of the honey against the damage induced by toxic agents is by apoptosis pathways, inflammation and oxidative stress (Samarghandian *et al*., 2022). The molecular mechanism of the honey protective effect was to downregulate the expression of COX-2 and NF-κB as inflammatory signals, and BAX and caspase-3 as apoptotic signals, while inducing Bcl-2 expression (Neamatallah *et al*., 2018). Honey stimulates the immune system as well as helps in wound healing (Medhi *et al*., 2008) Honey has cleansing action on wounds, stimulates tissue regeneration, and honey-

impregnated pads act as non-adhesive tissue dressing (Al-Waili, 2005, Bansal *et al.*, 2005 and Efem, 1988). Studies stated that honey treats many diseases that affect the liver, heart, blood vessels and gastrointestinal tract (Chowdhury, 1999).

ZnO-NPs is the most commonly-utilized group of high crystallinity-nanomaterials that are used in many industries where it added to ceramics, plastics, cement, glass, rubber, lubricants, paints, ointments, adhesives, pigments, sealants, batteries, ferrites, fire retardants (Hernández Battez *et al*., 2008). Studies have shown that ZnO nanoparticles produce cytotoxic effects in numerous kinds of cells, including hepatocytes, kidney cells, human bronchial epithelial cells, alveolar adenocarcinoma cells and osteoblast cancer cells (Kang *et al*., 2013). ZnO-NPs are easily absorbed to the bloodstream via the gastrointestinal tract, when administered as a single oral dose this property allows the liver, lung, and kidney to be the target organs for their accumulation and toxic effects (Cho *et al.* 2013 and Baek *et al*., 2012). ZnO-NPs could disturb the energy metabolism and cause mitochondria and cell membrane impairments in rat kidneys, which may contribute to ZnO-NPs induced nephrotoxicity. In spite of the fact that the increased use and production of ZnO-NPs may result in health impacts due to environmental contamination (Yan *et al*., 2012). Farghaly (2006) stated that the liver may suffer from extensive damage before malfunction so it is the organ most frequently studied to understand the changes of acute cell injury.

MATERIALS AND METHODS

1. Administration of Materials:

We use two doses of ZnO-NPs during the organogenesis period, the first is one low dose of 10 mg/Kg body weight of pregnant rats per day and the second is a high dose of 50 mg/Kg body weight of pregnant rats per day (Urekha *et al*., 2012). ZnO-NPs used with 2-10 nano-gram of particles size were suspended in distilled water forming suspension will be administered orally; the suspension was stirred for 10 seconds before administration. Honey is used orally during organogenesis period from the 6^{th} to 15^{th} day of gestation with a dose of 10 ml/kg body weight of pregnant rats per day (Kandil and Monir, 1986)

2. Experimental Animals:

Females and males of 11-13 weeks old weighing 120-180g were used for this study. Zero-day of gestation was determined by the presence of sperms in the vaginal smear at the estrus phase (McClain and Becker, 1975).

3. Experimental Design:

Pregnant females will be divided into six groups (n= 6-8, each) and each administrated orally during organogenesis period from the 6th to 15th day of gestation.

(C): was used as a control group and will receive distilled water.

(H): was administered with 10 mg/kg per day honey.

(ZnONPs10): was administered with ZnONPs with 10 mg/kg/day.

(ZnONPs50): was administered with ZnONPs with 50 mg/kg/day.

(H& ZnONPs10): was administered 10gm/kg per day honey before the receiving of ZnONPs (10 mg/kg/day).

(**H& ZnONPs50):** was administered10 mg/kg per day honey before the receiving of ZnO-NPs (50 mg/kg/day).

Animals of all groups were sacrificed on the 20th day of gestation.

1. Histological Examinations:

On the 20^{th} day of gestation, half numbers of the liver of pregnant rats of different groups were fixed in 10% neutral formalin buffer for at least one week then

washed with tap water and dehydrated by ethyl alcohol and then cleared in xylene, embedded in paraffin wax with series of changes, blocks were prepared for sectioning at 6 μm. Mount on clean slides, deparaffinised and stained by hematoxylin and eosin stains for histopathological examinations by the light microscope (Bancroft and Stevens, 1996).

2. Biochemical Parameters:

Some liver function tests are measured to determine aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Seral AST and ALT activity was determined according to the method described by Reitman and Frankel (1957) using a commercial reagent kit.

3. Electron Microscope Examination:

For TEM examination, about 1mm of the liver was fixed in gloteraldehyde at 4°C for 2 hours, rinsed in 0.1M phosphate-buffered and post-fixed in osmium tetra-oxide, then dehydrated by ethanol. After immersion in propylene oxide, the specimens were embedded in an epoxy resin mixture (Gupta, 1983). Ultrathin sections (60-90nm) were cut and picked up on copper grids; the sections were stained with uranyl acetate and lead citrate (Renolds,1963) and finally, stained sections were examined with a JEOL 1010 transmission electron microscope at the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University.

4. Statistical Analysis:

Results have been analyzed by prism version (5) programs. Comparison between the studied groups was carried out using the one-way ANOVA, where $P < 0.05$ was considered significant. All the values were presented as means ± standard errors of the means (S.E.M) (Armitage and Berry, 1987).

RESULTS

Histological Studies:

1-The Histopathological Changes in The Adult Liver:

Both of control and received honey groups of rats showed a normal structure and the histological changes in the livers of the studied groups were according to the dose of ZnONPs administration as follows:

A) The Group Received ZnONPs (10 mg/kg per day):

Livers of dams show pyknotic nuclei, hydropic degeneration, interstitial hemorrhage and central vein congested with blood (Fig.1 C). But when receiving Honey before ZnONPs (10 mg/kg per day) shows normal structure and cytoplasm of cells (Fig.1 D).

B) The Group Received ZnONPs (50 mg/kg per day):

Showing pyknotic nuclei, hydropic degeneration, congested BV and portal vein, interstitial hemorrhage and swelling cells (Fig. 1 E). But the administration of Honey before ZnONPs (50 mg/kg per day) shows a normal structure (Fig. 1 F).

1.2. The Histopathological Changes in The Fetal Tissue:

The control group showed some pyknotic nuclei, lymphocyte and erythrocyte but the group that received honey showed a normal structure. the differences in histological changes in the fetal tissue structure of the studied groups were according to the dose of ZnONPs that were maternally administered as follows:

A) The Group of Fetuses That Maternally Received ZnONPs (10 mg/kg per day):

The liver tissue on the 20th day of gestation showed pyknotic nuclei and hydropic degeneration (Fig. 2C). The group of fetuses that maternally received Honey and ZnONPs (10 mg/kg per day) showed some pyknotic nuclei but less than group (C) (Fig. 2D).

B) The Group of Fetuses That Maternally Received ZnONPs (50 mg/kg per day):

Showing interstitial hemorrhage, pyknotic nuclei and many vacuolated hepatocytes (Fig. 2 E). But the group of fetuses that maternally received Honey and ZnONPs (50 mg/kg per day) showed normal structure (Fig. 18 F).

Biochemical Parameters:

Effect of Honey on Liver Function Tests of Pregnant Rats Receiving ZnONPs:

The results are illustrated in Fig. (3). Aspartate transferase (AST) and Alanine transferase (ALT) normal values in the serum are 3.122±0.2490 and 1.827±0.1013, respectively. After administration of ZnONPs (by two doses) from day 6 to 15 of gestation, AST and ALT activities increased respectively as compared to the normal control group. Oral administration of honey before receiving ZnONPs decreased AST activities compared to the normal control group.

Transmission Electron Microscope Examination:

1. The Pathological Changes in The Adult Liver:

The group received ZnONPs (10 mg/kg per day) showing condensation of chromatin body, ER dilation, swolling to mitochondria, and irregular nuclear membrane (Fig.4 C). But the group that received honey before this dose showed a regular nuclear membrane and coherent nucleus (Fig.4 D).

The group received ZnONPs (50 mg/kg per day) showing vacuolation in the cytoplasm, ER dilation, nanoparticles precipitation on the nuclear membrane, accumulation of nanoparticles surrounding the nucleus, deformed mitochondria and irregular nuclear membrane (Fig.4 E). But the group received honey and ZnONPs (50 mg/kg per day) showing a regular nuclear membrane (Fig.4 F).

2.The Pathological Changes in The Fetal Liver:

The group received ZnONPs (10 mg/kg per day) showing an irregular nuclear membrane, and accumulation of nanoparticles in the nucleus (Fig.5 C). But the group received Honey and ZnONPs (10 mg/kg per day) showing a regular nuclear membrane and few nanoparticles on the nuclear membrane (Fig.5 D).

The group received ZnONPs (50 mg/kg per day) showing nanoparticle precipitation on the nuclear membrane, and inside the nucleus, deformed mitochondria and ER, and an irregular nuclear membrane (Fig.5 E). The group received honey and ZnONPs (50 mg/kg per day) showing and regular nuclear membrane (Fig.5 F).

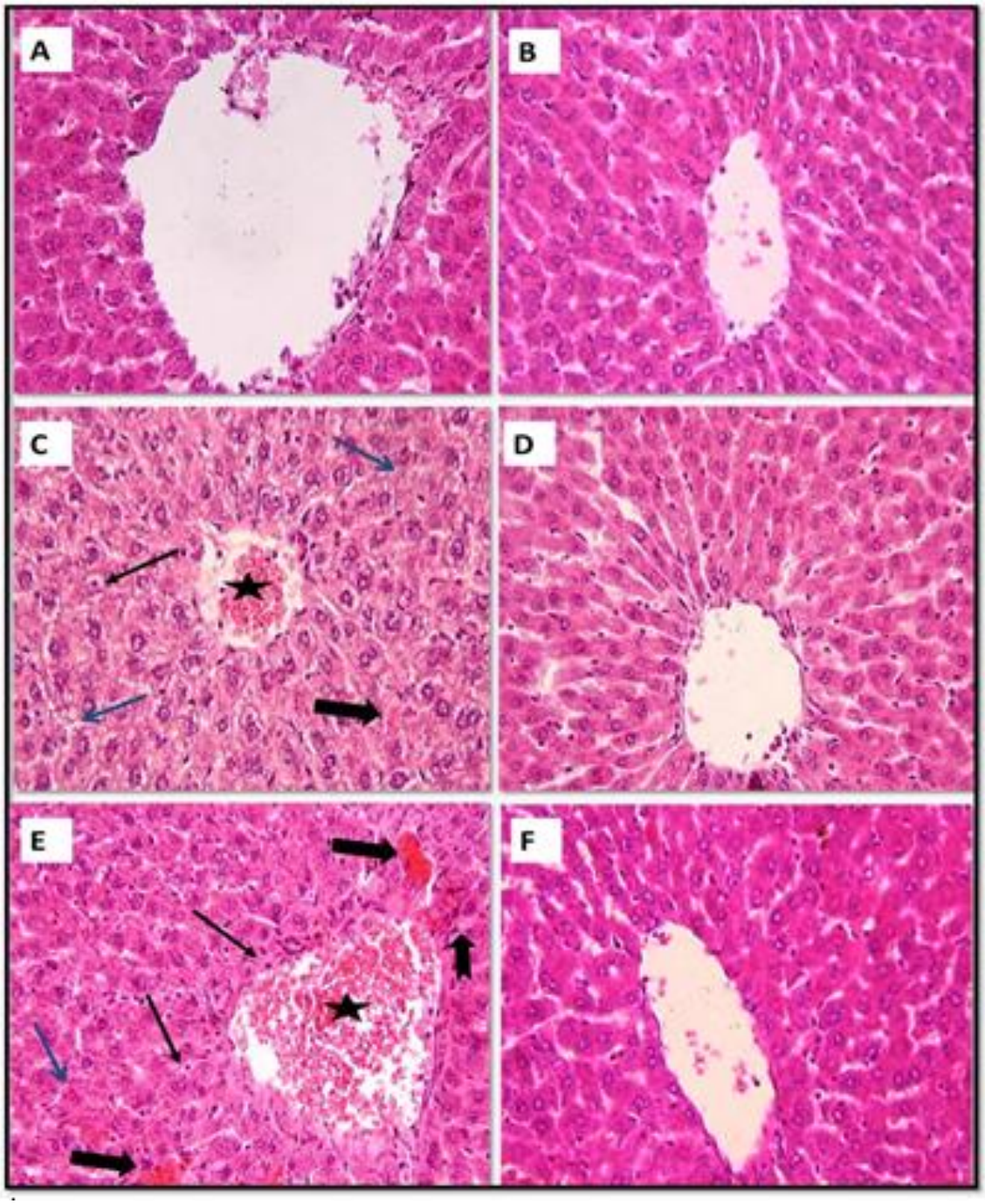

Fig. 1: Photomicrograph of liver of pregnant rats showing**:** (**A**) The control group received dist. H_2O and (**B**) received honey have a normal structure. The group received ZnONPs (10 mg/kg per day) (**C**) showing pyknotic nuclei (black arrow), hydropic degeneration (blue arrow), interstitial hemorrhage (wide arrow) and central vein congested with blood (star). The group received Honey and ZnONPs (10 mg/kg per day) (**D**) showing normal structure and cytoplasm of cells. The group received ZnONPs (50 mg/kg per day) (**E**) showing pyknotic nuclei (black arrow), hydropic degeneration (blue arrow), congested BV and portal vein (star), interstitial hemorrhage (wide arrow) and swelling cells (notched arrow). The group received Honey and ZnONPs (50 mg/kg per day) **(F**) showing normal structure. **(H&E, X.400).**

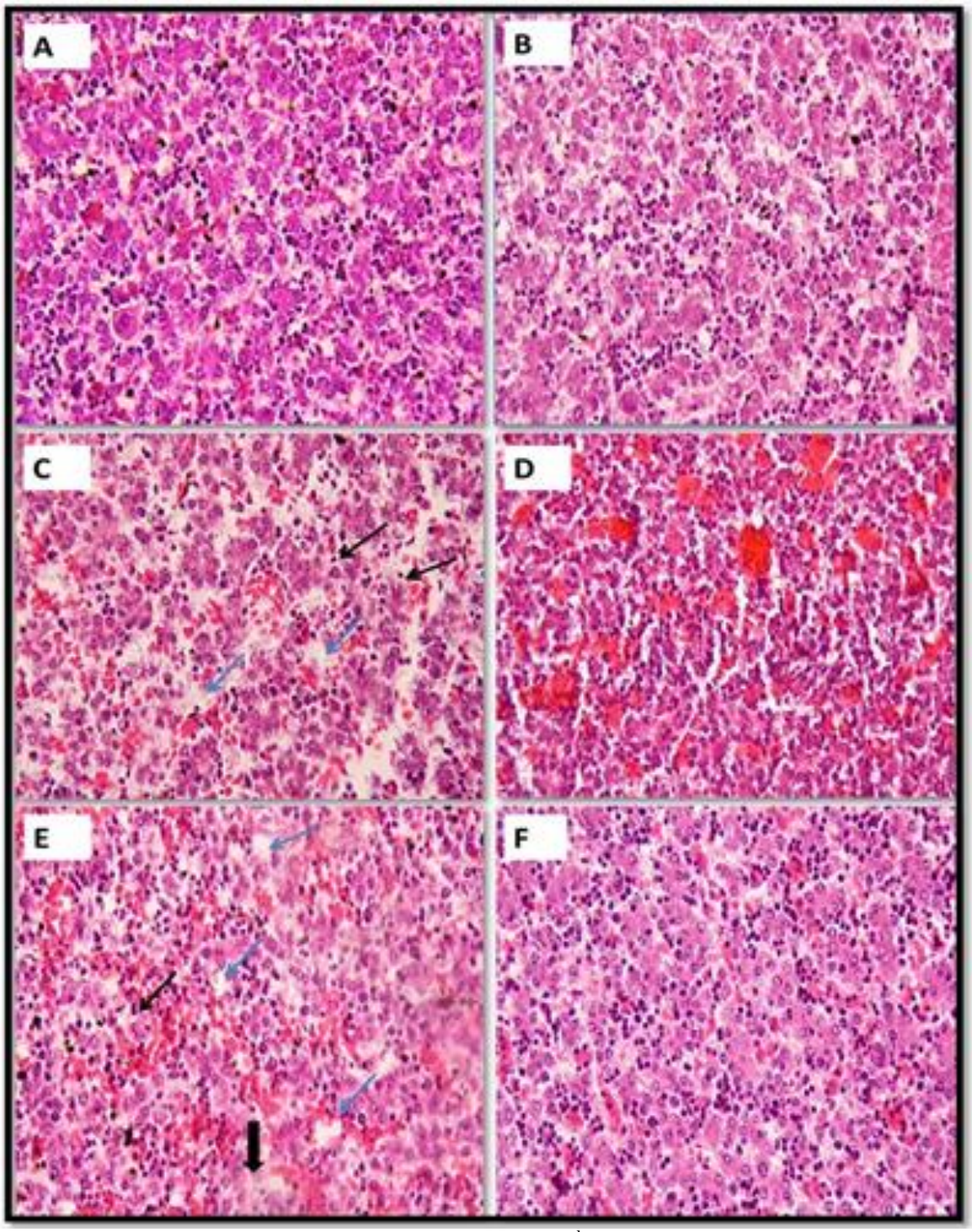

Fig. 2: Photomicrograph of the fetal liver at the 20th day of gestation: (**A**) The control group (maternally received dist. H2O) showing normal structure. (**B**) maternally received honey showing normal structure. The group of fetuses that maternally received ZnONPs (10 mg/kg per day) (**C**) showed pyknotic nuclei (black arrow) and hydropic degeneration (blue arrow). The group of fetuses that maternally received Honey and ZnONPs (10 mg/kg per day) (**D**) showed some pyknotic nuclei but less than group (C). The group of fetuses that maternally received ZnONPs (50 mg/kg per day) (**E**) showed interstitial hemorrhage, pyknotic nuclei (black arrow) and many vacuolated hepatocytes (blue arrow). The group of fetuses that maternally received Honey and ZnONPs (50 mg/kg per day) (**F**) showed normal structure**. (H&E, X.400).**

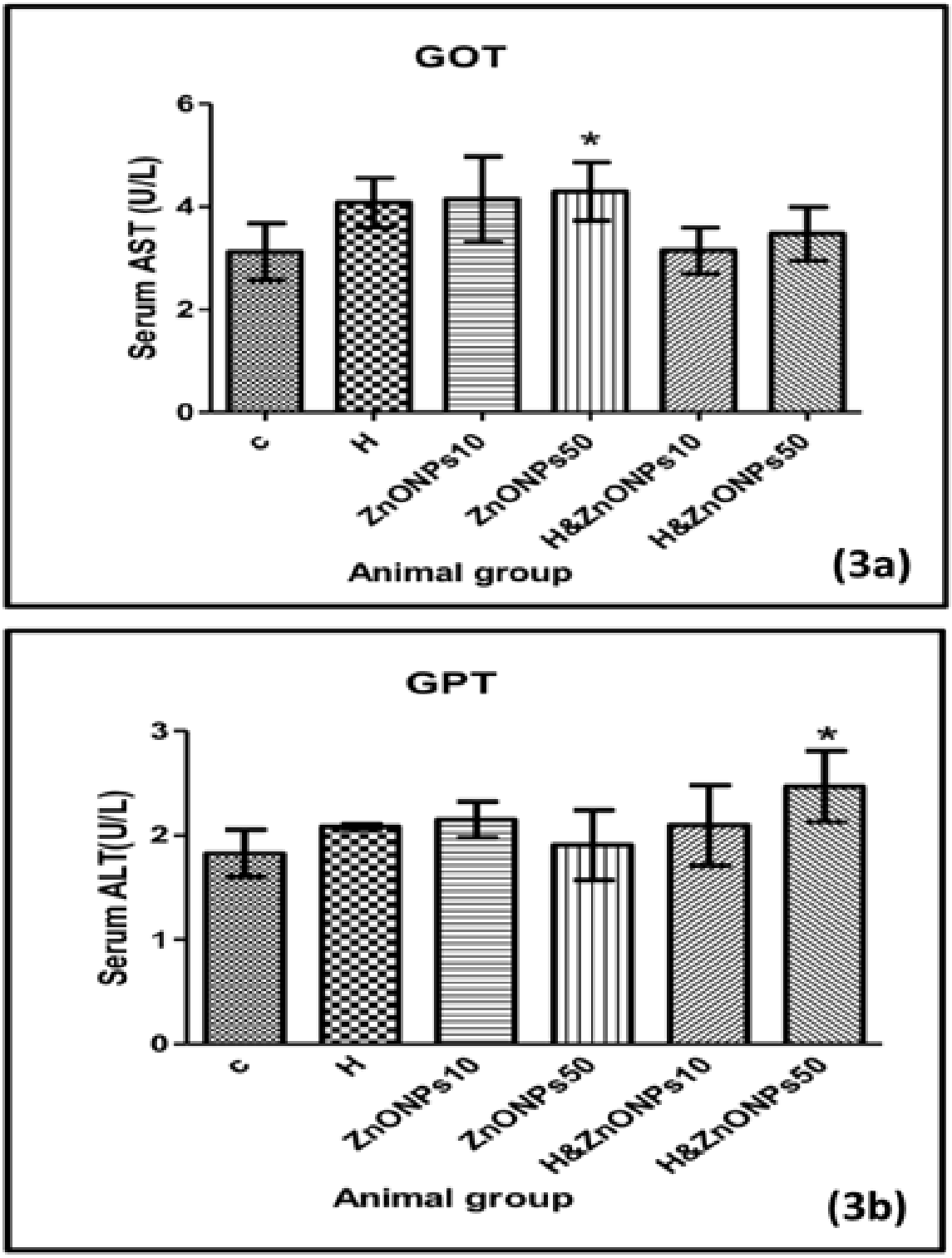

Fig. (3a&3b): Effect of Honey on AST& ALT activities of Pregnant Rats Receiving ZnONPsStatistical analysis was carried out by One Way ANOVA followed by Tukey-Kramer Multiple Comparison Test. C=Normal Control, **H**= honey, **ZnONPs10**= ZnONPs (10 mg/kg per day), **ZnONPs50**= ZnONPs (50 mg/kg per day), **H&ZnONPs10**= ZnONPs (10 mg/kg per day) and honey, **H&ZnONPs50**= ZnONPs (50 mg/kg per day) and honey. * Significantly different from normal control group at $P < 0.05$.

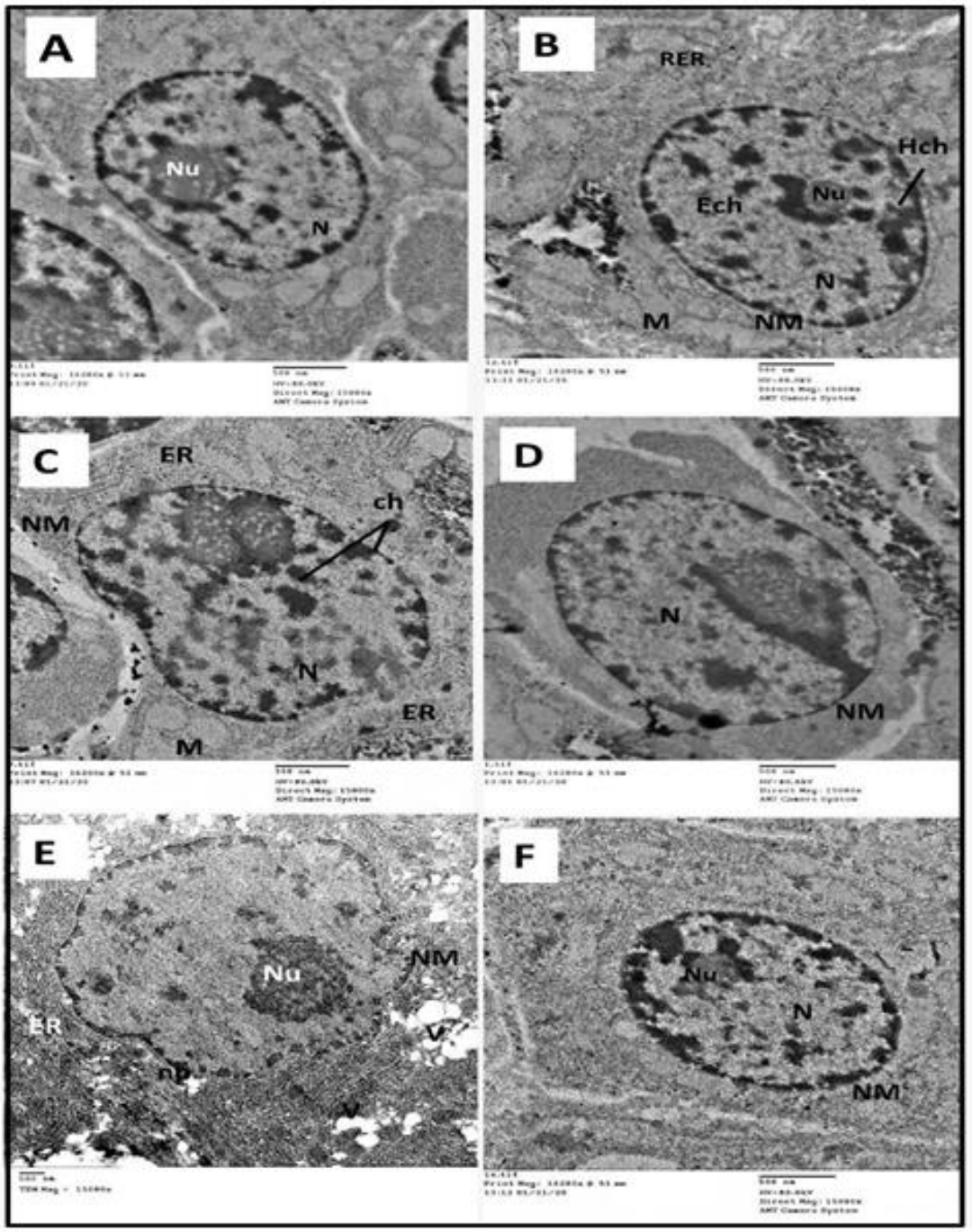

Fig. 4: Photomicrograph of liver of pregnant rats showing**:** The control groups (**A**) received dist. H_2O and (**B**) received honey, and have normal architecture. The group received ZnONPs (10 mg/kg per day) (**C**) showing condensation of chromatin body, ER dilation, swolling to mitochondria, and irregular nuclear membrane. The group received Honey and ZnONPs (10 mg/kg per day) (**D**) showing regular nuclear membrane and coherence. nucleus. The group received ZnONPs (50 mg/kg per day) (**E**) showing vacuolation in the cytoplasm, ER dilation, nanoparticles precipitation on the nuclear membrane, accumulation of nanoparticles surrounding the nucleus, deformed mitochondria, and irregular nuclear membrane. The group received Honey and ZnONPs (50 mg/kg per day) **(F**) showing a regular nuclear membrane. **(TEM, X.8000:15000)**

*__N__: Nucleus, **Nu**: nuclear membrane, **M**: Mitochondria, **Ech**: Euechromatin, **Hch**: Heterochromatin, **RER**: Rugh Endoplasmic Reticulum, **np**: Nanoparticles, **V**: vacule

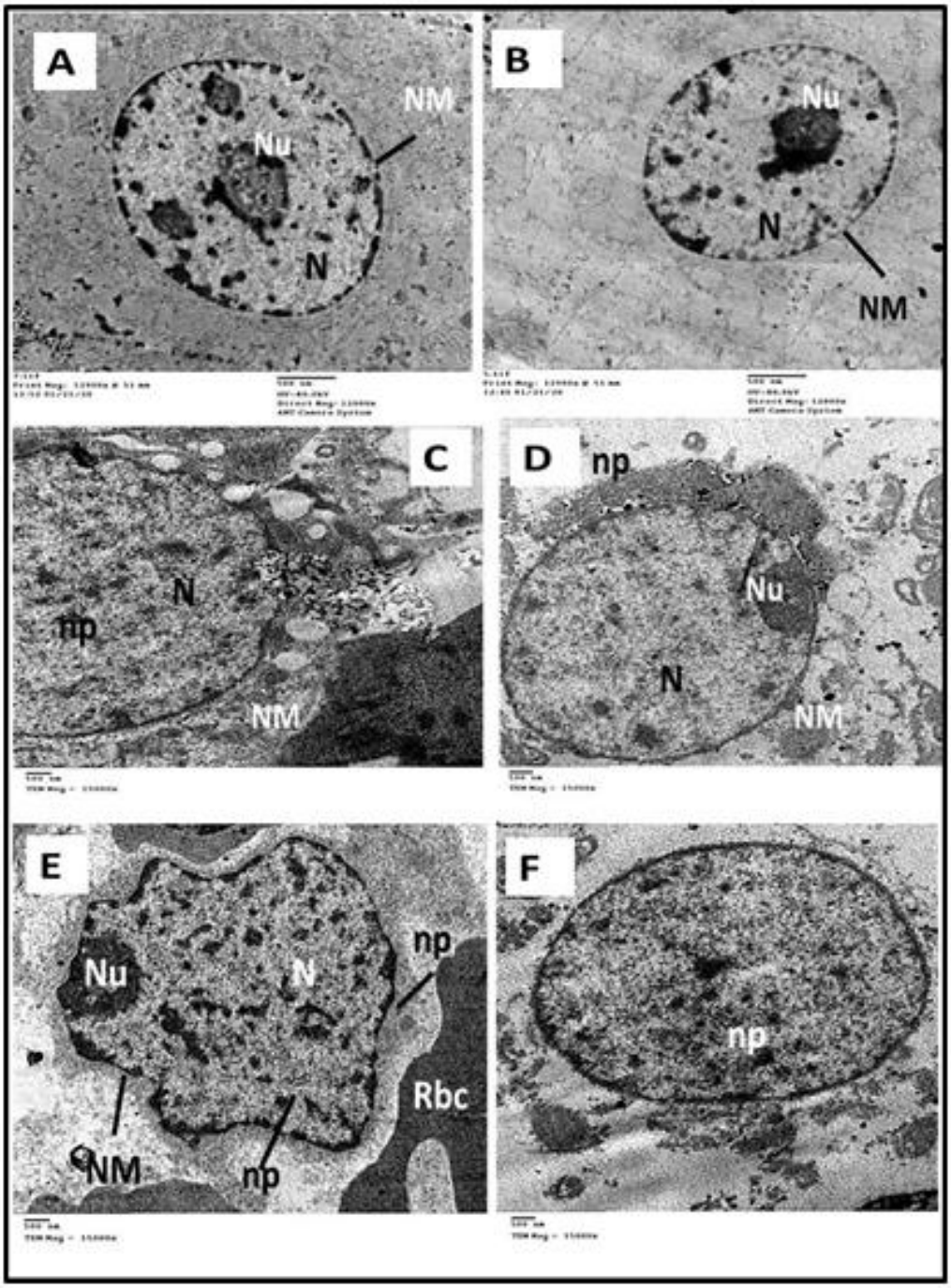

Fig. 5: Photomicrograph of fetal liver showing**:** The control groups (**A**) received dist. H_2O and (**B**) received honey, and have normal architecture. The group received ZnONPs (10 mg/kg per day) (**C**) showing an irregular nuclear membrane, and accumulation of nanoparticles in the nucleus. The group received Honey and ZnONPs (10 mg/kg per day) (**D**) showing a regular nuclear membrane and few nanoparticles on the nuclear membrane. The group received ZnONPs (50 mg/kg per day**)** (**E**) showing nanoparticle precipitation on the nuclear membrane, and inside the nucleus, deformed mitochondria, and irregular nuclear membrane. The group received Honey and ZnONPs (50 mg/kg per day) **(F**) showing a regular nuclear membrane. **(TEM, X.15000:20000)**
*__N__: Nucleus, **Nu**: nuclear membrane, **M**: Mitochondria, **Ech**: Euechromatin, **Hch**: Heterochromatin, **RER**: Rugh Endoplasmic Reticulum, **np**: Nanoparticles, **V**: vacule

DISCUSSION

The liver is one of the major organs in the body which are responsible for the removal of toxins and poisons. Examination of transverse sections of maternal and fetuses maternally administered orally during the organogenesis period showed marked histopathological changes in the liver. This study agrees with the study of Hua-Qiao *et al*. (2016) where it was proved that ZnO-NPs Induce pathological changes in the liver**.**

The histopathological changes in dams and their fetuses maternally received ZnO-NPs were related to the vascular system, where dilation and congestion in the portal vein or central vein, lymphocytic infiltration, interstitial hemorrhage and hepatic sinusoid and the hepatocyte showed degenerative changes in pregnant rats and their fetuses. This was proved by a previous study that identified the toxicity of ZnO NPs, including liver damage, cytotoxicity, membrane injury, and inflammatory response (Sharma *et al*, 2012 and Esmaeillou *et al*, 2013).

Hua-Qiao *et al*. (2016) reported that ZnO nanoparticles change the indices of hematology and blood chemistry. Many studies have reported that excess oral administration of zinc salt and zinc powder can lead to liver damage and liver dysfunction, with increased values of serum enzymes such as glutamic–pyruvic transaminase/glutamic–oxaloacetic transaminase and alkaline phosphatase. The present study agrees with these studies where the percentage of these serum enzymes increased aid primarily in the diagnosis of liver disease or damage.

ZnO nanoparticles may induce the formation of highly reactive oxygen species, including hydrogen peroxide, hydroxyl radicals, and superoxide anions, all of which can cause oxidative damage to animal cells (Sharma *et al*, 2012).

When administered as a single oral dose, ZnO nanoparticles are easily absorbed into the bloodstream via the gastrointestinal tract, and this property allows the liver, lung, and kidney to be target organs for their accumulation and toxic effects (Cho *et a*l., 2013 and Baek *et al* 2012). in our study, this accumulation and toxic effects appeared clearly in liver tissues that administered ZnONPs

In our study, the use of honey decreases the side effects of ZnONPs. Chowdhury, (1999) observes that honey treats many diseases that affect the liver, heart, blood vessels and gastrointestinal tract. And inhibit free radical formation, thus showing anti-inflammatory effects (Markelov and Trushin, 2006).

CONCLUSION

Collectively, ZnO-NPs were developmentally toxic to pregnant rats and their fetuses as evidenced by the pathological changes in livers and the defect in Liver Function of pregnant rat and their fetuses but administration of honey during pregnancy decreased these effects.

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