How to Cite:
Hussin, W. S. A., Obeid, A. K., & Jawad, R. A. A. (2022). The protective effect of aqueous of Moringa Oleifera seeds extract against levofloxacin drug-induced liver damage in male white rats. *International Journal of Health Sciences*, *6*(S2), 13044–13052. https://doi.org/10.53730/ijhs.v6nS2.8480

The protective effect of aqueous of Moringa Oleifera seeds extract against levofloxacin drug-induced liver damage in male white rats

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***Abstract*---**Objective The goal of this study is to find out how effective Moringa seed (Moringa oleifera) aqueous Extract is in protecting liver tissue from oxidative stress caused by levofloxacin drug . Methods: 24 male albino rats were placed into four groups at random, with six rats in each group being treated for 30 days: Group 1 was a control group, whereas Group 2 was given levofloxacin at a dosage of 10 mg/kg orally by gavage. , The third group received aqueous extracts of Moringa seeds 650 mg/kg, while the fourth group received aqueous extracts of Moringa seeds 650 mg/kg dose before 4 hours of receive orally at a 10 mg/kg per day dose . Results: The albino rat was considerably protected from levofloxacin-induced liver damage when given the aqueous extract Moringa seeds orally at a concentration of 650 mg/kg body weight. The action of the extract also protected the liver damage caused by the medication, which was indicated by congestion central vein degeneration hepatic cell, necrosis hepatic cell, irregular hepatocyte organization, dilatation and congestion of portal vein , Expandation of sinusoids and increased infiltration of inflammatory cells on histological examination. Conclusion: the aqueous extract of Moringa seeds strengthens oxidative stress defenses against hepatotoxicity.

***Keywords*---**Moringa Oleifera seeds, histopathology, liver, levofloxacin drug.

Introduction

Medicinal plants are among the things that have increased interest in the current century by the World Health Organization WHO, despite the development in the fields of chemistry and pharmacology because these chemical drugs have a negative effect that appears after a long period of treatment (am Ende & am Ende, 2019) *Moringa Oleifera* is a part of the Moringaceae family species. Its leaves are rich of macroand micro-nutrients including, vitamins, phenolic acids, carotenoids, flavonoids, and alkaloids, polyphenols, minerals (Liang *et al.*, 2020) Therefore, Moringa Oleifera plant is used in nurturing of human as an excellent nutritive supplement (Sanjay & Dwivedi, 2015),the Moringa tree nutritional and medical importance have been used for ages in treating many different diseases in conventional medicine, such as anti-hypertension, heat, diabetes, fats, cardiac stimulant, circulatory, immunology, antioxidant, tumors, infections, ulcers, depression, bacteria, fungi, cramping, aging, diuretic, and liver diseases(Jahan, *et al.*, 2018) .The seeds of M. Oliefera are nutritionally important as they are freshly eaten largely green or as ground seed in the northern part of Nigeria (Zade *et al.*, 2013). The percentage of protein in the seeds is high, as the dried seeds contain 18-25% of protein, which is almost twice the amount found in the grains .., and effective plant components were also observed in the seed extract of Moringa Olivera, such as alkaloids, flavonoids, steroids, and phenols. Phenolics, tannins, saponines (Ogbunugaf ,*et al.,*2011 :Ma *et al.*2020). Examination of these components helps to reassess the chemical components of the plant and which one prevails over the other. It also helps to search for biologically active agents such as its launch of a product that is partially used in some useful medicines and thus it is considered the most important seed Legumes for human nutrition, as there are many saturated fatty acids, including arachidic acid, stearic acid, palmitic acid and benic acid, in addition to that it contains the most important unsaturated fatty acids Oliec acid, as it reaches a high percentage (67.9 - 70.0%) (Ogbunugaf ,*et al* 2011) . The moringa plant contains many active substances, the most important of which are flavonoids, that play a role In curbing oxidative stress that results from the generation of free radicals as well as protecting the body from cancer and heart diseases (Gopalakrishnane *et al.*, 2016) . And flavonoids also improve the human protective enzyme system and protect it from diseases associated with aging (Hassan et al ., 2020). It provides protection for the human body from oxidative stress resulting from the use of drug (Anwar *et al.*,2007; Dubey *et al.*, 2013) M.*Oliefera* relatively contains high antioxidant activity in its leaves, flowers, and seeds .The extracts of M.*Oliefer*a both mature and tender leaves have potent antioxidant activity against free radicals, prevent oxidative damage to major biomolecules and afford significant protection against oxidative damage)

Materials and Methods

Experimental animals

The total number of animals used were 24 males rats obtained from Pharmacy College, University of Karbala, Iraq. Mean weight of subjects was 180 -220 gm and mean ages was 3-4 months. Subjects were preserved at a temperature (22-28℃), Light and 12hrs. dark cycle throughout the entire experimental period. and were given food and water ad libitum. All male rats were randomly divided into four main groups(6 rats/group): G1: Consider as a control group, G2 : rats dosed Levofloxacin orally with 10 mg/kg concentrations per day , G3 : included animals dosed orally with aqueous extract of *Moringa oleifera* seeds at a concentration of 650 mg/kg of body weight per day , G4 : was oral administered *Moringa Oleifera* seeds extract at a 650mg/kg dose before 4 hours of receive ii orally at a 10mg/kg per day dose, All treatment were carried out for 30 days .

Preparation of *Moringa Oleifera* Seeds

Dry seeds of *Moringa Oleifera* were obtained from the local market of kerbala governorate, Iraq . The seeds were cleaned and dried for 3 days at room temperature, the dried seed were milled to fine powder using a mechanical grinder A 20 g. of dry powder was taken and blending with 200 ml of distilled water, for 24 hourd at room temperature, the solution was filtered. the extract was dried in the oven after placing it in sterile glass dishes at 30oC for 24 (Hernandez et al., 1994). The concentrated extract was stored until use for this study.

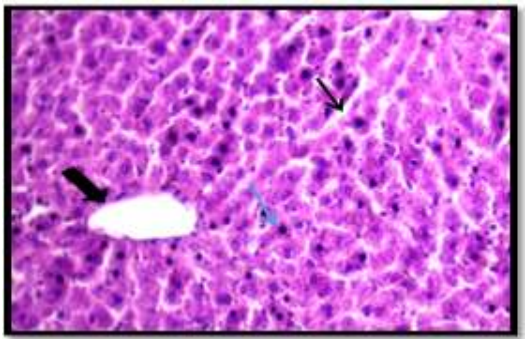
Tissue Sampling

The tissue samples collected from the Liver of all the rats , from all groups were anaesthetized with chloroform , Immediately after death the Liver was excised and fixed in 10 % formalin solution for 48 hours. The samples then dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin wax at 56 C in oven and made as blocks. The blocks were carefully oriented to have the cross in microtome. 5 µm thickness serial sections were cut. The sections were deparaffinied and hydrated for Hematoxylin and Eosin (Suvarna *et al.*, 2013; Obeid *et al.*, 2020).

Results and Discussion

The results of histological examination showed that Liver section of control group in Figure (1) showed the normal tissue structure , in which normal central vein , Sinusoids, Hepatocytes .Fig (2) , (3) ,(4) also Levofloxacin rat group showed histopathological changes including dilatation and congestion of central vein, degeneration hepatic cell ,necrosis of hepatic cell , irregular arrangement of Hepatic cord , increased infiltration of inflammatory cells, Expandation of sinusoids and dilatation and congestion of portal vein , inflammatory cell infiltration in the periportal area Compared with control groups (Fig 1). As for the histological examination of the Liver of aqueous extract of *Moringa Oleifera* at concentrations of 650 mg/kg for a period of 30 day in Fig (5) show normal

appearance of central vein , Sinusoids , Hepatic cell. Fig (6) section of rat rats received 650 mg /kg of *Moringa Oleifera* seed extract with Levofloxacin 10 mg/kg showing nearly normal of central vein , Sinusoids, Hepatocytes Compared with Fig (2,3,4).



Figure(1): Liver section of rat (control) Showing normal appearance of central vein (thick arrow) , Sinusoids(thin arrow) , Hepatocytes (blue arrow) (H&E 200X)

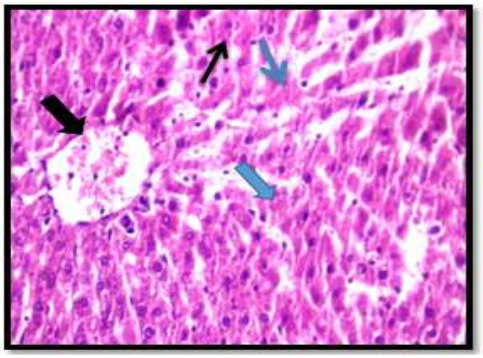


Figure (2) : Liver section of rat, treated group with Levofloxacin10 mg/kg showing that dilatation and congestion of central vein ((thick arrow) degeneration hepatic cell (thin arrow),necrosis of hepatic cell (blue arrow) ,irregular arrangement of Hepatic cord (thick blue) , (H&E 200X).

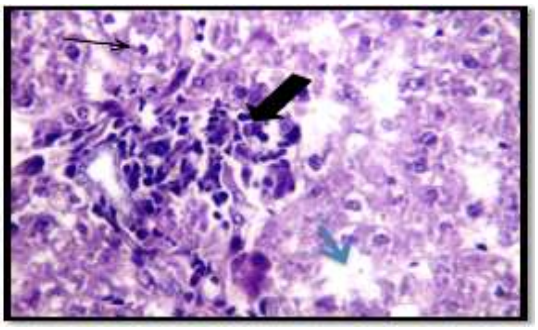


Figure (3) : Liver section of rat, treated group with Levofloxacin 10 mg/kg showing that increased infiltration of inflammatory cells(thick arrow) degeneration hepatic cell (thin arrow), Expandation of sinusoids (blue arrow) (H&E 400X)

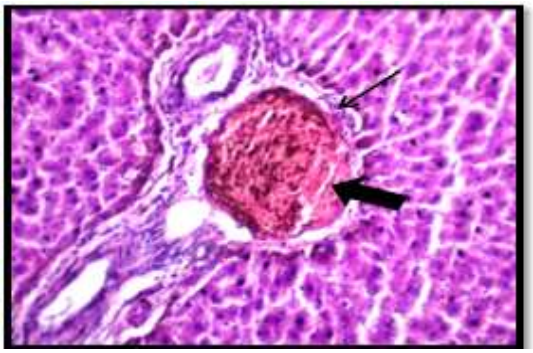


Figure (4) : Liver section of rat, treated group with Levofloxacin 10 mg/kg showing that dilatation and congestion of portal vein (thick arrow), inflammatory cell infiltration in the periportal area (thin arrow) (H &E 200X)

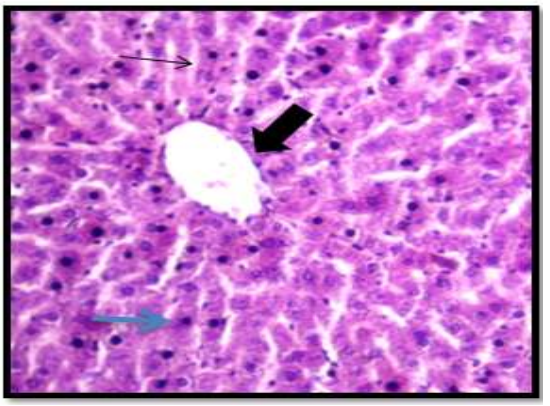


Figure (5) : Liver section of rat, treated group with aqueous extracts of Moringa Oleifera seeds 650 mg /kg showing that normal appearance of central vein (thick arrow) , Sinusoids (thin arrow) , Hepatic cell (blue arrow) (H&E 200X) .

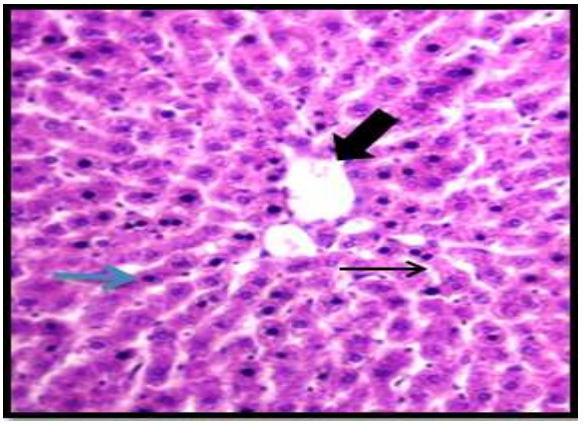


Figure (6) :Liver section of rat, treated group with aqueous extracts of Moringa Oleifera Seeds 650 mg /kg and Levofloxacin 10 mg/kg showing nearly normal of central vein (thick arrow) , Sinusoids(thin arrow) , Hepatocytes (blue arrow) (H&E 200X)

Discussion

The histological study, The hepatic histological examination of the positive control group (G2) treated with Levofloxacin 10 mg/kg for a period of 30 day revealed dilatation and congestion of the central vein, hepatic cell degeneration, hepatic cell necrosis, and an abnormal arrangement of the hepatic cord in Fig. (2), (3),(4) , This agrees with (Nada &Shawi 2012) (Ravikumar *et al.*,2020) . Where studies indicated that the mechanism of hepatotoxicity caused by fluoroquinolones, including levofloxacin drug, is not yet understood, it may be attributed to the formation of free radicals, which are oxidative factors that contribute to mitochondrial damage, thus inhibiting cellular respiration and reducing energy production by blocking the chain of electron transport in the mitochondria, Free radicals cause oxidative stress by stimulating the oxidation of lipid molecules in cell membranes as well as other essential components inside cells such as nucleic acids, proteins, and others, resulting in the programmed death of cells in other tissues (Hsiao et al., 2010) , Other research has found that this medicine increases tissue toxicity by blocking natural antioxidants in cells, inducing oxidative stress in renal and hepatic tissue cells in rat (Ayokanmi and Olaniyi, 2015) (Alaa and Naseer, 2021).

Fluoroquinolones have been shown to be hepatotoxic (Clark.,2001). The formation of reactive oxygen species by fluoroquinolones caused cellular damage in the liver and kidney, according to studies (Xiao,2010) . The activation of signaling pathways that promote increased mitochondrial generation of free radicals and lipid peroxidation, causes hepatocyte injury (Tilg and Diehl, 2000). As for the histological examination of the liver in group (G3) in figure (5), the group that was dosed with *M. Oleferia* extract at a concentration of (650) mg / kg the absence of any changes in the tissue indicating the presence of a disease or an apparent defect as the tissue appears normally of the central vein, Sinusoids and hepatocytes Compared with group (G1) in Fig. (1) *, M .Oleifera* is a plant that is found in the Oleifera family Antioxidants operate in the detoxification mechanism and are one of the most significant variables for eliminating free radicals and reducing the consequences of oxidative stress (Ahmed *et al.* 2018) The Ethanolic extract of Moringa Oleifera at doses (400mg/kg. 600mg/kg and 800mg/kg) on the liver did not show any visible lesion or disease which implies that administration of M. Oleifera is non-toxic to animals at low and high doses (Ekundina *et al.,*2015)(Shatha and Naseer , 2020).

The antioxides includes glucosinolates, such as gluco-moringin, flavonoids, such as quercetin and kaempferol, and phenolic acids, such as chlorogenic acid (Yassa and Tohamy, 2014) (Kasolo *et al.*, 2010) The histological examination of the liver in group (G4) shows in Figure (6) the near-normal appearance of the central vein and the regularity of the hepatic cords with slight expansion of the sinuses and the central vein This agrees with (Taha *et al.*, 2015) As a result of the protective role of Moringa seed extract against oxidative stress and the resulting lipid peroxides. *M. Oleferia* seed extract protects against oxidative stress and the lipid peroxides that occur .agree with this study (Liang *et al.*,2020) .

The presence of *M. Oleferia* Seeds for a number of phenolic, flavonoids compounds, and vitamins, including ascorbic acid and tocopherol, glycosides,

tannins, and terpenoids, which act as free radical scavengers and reactive oxygen species (ROS), thus working to inhibit oxidative damage and prevent fat peroxidation (Shailaja *et al.*,2008). compounds quench of ROS, and regenerate membrane-bound antioxidants. This finding is consistent with previous studies which demonstrated the antioxidant activity of *Moringa* extract (Kumar & Pari, 2003) . *M. Oleifera* seeds, fruits, and leaves have been discovered to contain antioxidants. When comparing the antioxidant performance of M . Oleifera seeds to that of palm oil, *M. Oleifera* seeds were shown to be the most effective root sweep strategy of oxygen radicals (ROS). (Ogbunugafor ., 2011) (MARZA *et al .,* 2021) . According to(Mahajan *et al* ., 2008) , the reduced creation of reactive oxygen species (ROS) in the *M. Oleferia* extract group resulted in less tissue damage, owing to the efficiency of high seed extract in reducing the development of the sources of oxygen radicals (ROS)(KAREEM and OBEID, 2020).

Conclusions

We conclude from this that the aqueous extract of *Moringa Oleifera* seeds has a positive effect in the liver in male rats, as well as highly effective in antioxidants, and reduces the toxic effect and tissue changes caused by Levofloxacin

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