Histological Study Of Protective Effects Of The Aqueous Extract Of *Moringa Oleifera* Seeds On Diclofenac-Induced Livers In Male Rats

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**Abstract:** The study was designed to indicate the protective effect of the hot aqueous extract of *Moringa oleifera* the seeds against the harmful effects stimulated by the diclofenac drug in the livers of the male rats. This study was carried out in the animal house of Science College / Department of Biology - University of Kufa from 1/10/2022 to 10/1/2023. Thirty male rats weighted (220-225) g and ages of (11-12) weeks were divided into 6 groups of 5 rats of each set which were treated orally for 35 days and were as: 1 group was provided with water only(control), while the 2 group was dosed with only diclofenac of (100) gram/kilogram/b.m. 3 set was treated with water seed extract of *Moringa oleifera* of (450) gram/kilogram/b.m., 4 set was treated with water seed extract of *Moringa oleifera* of (600) gram/kilogram/b.m., while 5 set was treated with water seed extract of *Moringa oleifera* of (450) gram/kilogram/b.m. + diclofenac, and the last set 6 was treated with water seed extract of *Moringa oleifera* of (600) gram/kilogram/b.m. + diclofenac, animals were dosed of (35) day, then they were sacrificed and livers were extracted and fixed in formalin 10% for 48 hours for histological study. Results showed there were no any pathological changes in the histological structure of rat livers treated with water seed extract of *Moringa oleifera* of (450, 600) gram/kilogram/b.m., as results also of the set treated with water seed extract of *Moringa oleifera* of (450,600) gram/kilogram/b.m. + diclofenac indicated liver tissue was closer to normal structure with slight changes, while results of rat liver tissues treated with diclofenac had suffered from pathological changes such as central vein expansion, sinusoids widening, necrosis and degeneration of liver cells and filtering of inflammatory cells compared with control set.

**Keywords:** aqueous extract ,*Moringa oleifera* ,seeds , diclofenac, livers ,male rats.
1. Introduction

Diclofenac is an anti-inflammatory drug that belongs to the non-steroidal anti-inflammatory drug class. It was first approved by FDA in 1988 under the trade name (Voltaren) and marketed by Novartis [1]. It works by reducing the production of prostocladin and oxidation enzymes that cause inflammation and pain in the body. This drug is one of the most widely used drugs in all parts of the world. Pain-relieving anti-inflammatory drugs are among the most common medications that help relieve pain quickly, and they can be used Over the counter for fever and pain relief and to treat mild to moderate pain, rheumatoid arthritis, spondylolisthesis, eye infections, fever, gout, and migraine [2].

Diclofenac absorption is rapid and complete when given orally, and this drug is highly bound to plasma albumin [3]. It is completely absorbed by the gastrointestinal canal, but it may undergo primary pass metabolism of only 60% of a drug which reaches to systemic circulation passing unnoticed [4]. The mechanism of action of diclofenac is summarized in its ability to inhibit the enzyme cyclooxygenase COX, which helps in the formation of chemicals known as prostaglandins that cause pain and inflammation. Preventing the formation of this substance leads to a reduction in pain and inflammation, as diclofenac inhibits the manufacture of the cyclooxygenase 2 enzyme and thus reduces the incidence of infection. About the side effects of the digestive system, compared to both indomethacin and aspirin, the effectiveness of a single dose of diclofenac (6 to 8 hours) is much longer than the half-life of the drug, which is considered short [5]. The reason for this can be attributed to the fact that diclofenac persists for more than 11 hours in the synovial fluid.

Diclofenac inhibits the synthesis of cyclooxygenases (1,2), and these enzymes are responsible for the synthesis prostaglandins (PGs). It has significant activity in pain and inflammation and inhibition of its production is the important drug action that links all effects of diclofenac [6]. Many serious side effects of diclofenac include cardiovascular thrombosis, heart failure, high blood pressure, sore throat, burning eyes, skin rash, exfoliative dermatitis, idiopathic use of many analgesics, renal failure, and heart failure, leading to death even at the treated dose [7; 8].

The Moringa oleifera plant is one of the important medicinal plants that has been used as a treatment for many diseases and as a substitute for many drugs and medical medicines. This plant has been used since ancient times because it contains organic substances such as amino acids, phenols, carotenoids, flavonoids, isothiocyanates, enzymes, glucosins, minerals, sterols, and tannins, which are substances responsible for the formation of antioxidants in the human body. These substances contain many properties, such as hepatoprotective properties, antioxidants, and antimicrobials [9].

In recent times, Moringa oleifera has entered medical laboratories as a healthy nutritional supplement to reduce malnutrition [10]. It has also been used in the treatment of cardiovascular diseases, antihypertensives, protection against diabetes, cancer, oxidation, tumor treatment, spleen enlargement, infections, convulsions, depression, germs, and fungi [11]), as for the seeds and flowers of this plant, they are anti-allergic and reduce fat in the body, while the roots are anti-inflammatory and the bark is anti-ulcer [12]. This is done by removing organic impurities and turbidity because it is easy to implement, its cost is low, and it does not contain chemicals [13], as well as being used in the manufacture of soap, shampoo, and cosmetics by using the saturated fatty acid present in it [14], and the reasons for using moringa in the manufacture of cleaning materials. It is effective in controlling germs, microbes, and disease-causing parasites that are transmitted by contaminated hands [15]. The study aims to indicate the protective effect of w
water seed extract of *Moringa oleifera* towards the histological effects stimulated by diclofenac of male rat livers.

2- Methodology

This study was conducted in the animal house of the College of Science/University of Kufa for the period from 11/2022 to 12/2023.

**Animals**

The total number of adult male rats used in this study was 30, with a weight between (220-225) g and an age between (11-12) weeks. The rats were placed in plastic cages covered with metal covers, and the floor was covered with fine wood sawdust. The cages were cleaned and the floor was constantly replaced, and they were sterilized with disinfectants. Rat animals were placed under similar laboratory conditions in terms of temperature (20 to 25 °C), lighting (12 hours per day), and good ventilation. The animals were also provided with water and a diet freely throughout the research period, and the rats were left to adapt for 2 weeks before conducting research and making sure that it was free of diseases [5].

**Preparation of water seed extract of *Moringa oleifera***

preparation of hot water seed extract of *Moringa oleifera* by purchasing them from shops selling herbs in the province of Najaf, which were classified by professors specialized in plant classification at the University of Kufa, then these seeds were ground with a laboratory medicinal herb grinder until a fine powder was obtained. (20) gram of powder of dry seeds was taken and milled with (400 ml) of hot distilled water by mixer and left for 24 ours under room temperature, The jumbles were filtered by layers of medical muslin of purpose getting rid of plankton and after that centrifuged at 3000 rpm for 10 minutes. After that, the extract was filtered with filter papers (Whatman- No. 101) for obtaining a pure solution and then dried in the oven after being placed it in glass petri dishes and sealed until use [16].

**Doses of Diclofenac**

The dose of diclofenac required in the current study was prepared after obtaining it, which was in the form of pills of a dose of (100) mg, which is a therapeutic dose in humans. Then, the dose that was given to animals according to their body weight was prepared.

**Experimental animals Division**

The number of rats used in the current study was thirty male rats, which were divided randomly into six sets, each set containing five animals, and they were treated as follows:
The first set, (control set), was given only distilled water.

The second set: was dosed only with diclofenac of 100 gram/kilogram /b.w.

The third set: was dosed with a hot water seed extract of *Moringa oleifera* of 450 gram/kilogram /b.w.

The fourth set: was dosed with a hot water seed extract of *Moringa oleifera* of 600 gram/kilogram /b.w.

The fifth set: was dosed daily with a hot water seed extract of *Moringa oleifera* of 450 gram/kilogram /b.w. for two hours before they were given diclofenac of 100 gram/kilogram /b.w.

The sixth set: was dosed daily with a hot water seed extract of *Moringa oleifera* of 600 gram/kilogram /b.w. for two hours before they were given diclofenac of 100 gram/kilogram /b.w. The animals are fed daily in one dose by a gastric tube device for 35 days.

**Animal anatomy**

Male rats were dissected after 35 days of treatment, as the animals were anesthetized with chloroform. Then the animals were fixed to the dissection dish with staples, and the abdominal cavity was opened longitudinally with sharp scissors. Then the livers were extracted from the animals after removing the connective tissue from them, and they were
washed with water and then dried with filter paper. After that, the livers were cut and placed in a formalin solution (10%) for 48 hours to prepare histological sections from them [8].

Preparation of histological sections of the livers.

Histological sections of male rat livers were prepared according to the method of [17].

Examination and Photomicrography of Microscopic sections

Microscopic sections of livers were examined to identify histological changes using a compound-type microscope (Olympus CX21) and under force (40X), and histological sections were also photographed by an imaging microscope after it had been equipped with a digital camera connected to the same computer.

3-Results and Discussion

The results of the examination showed the histological sections of the liver tissue of rats treated with water seed extract of Moringa oleifera of (450, 600) gram/kilogram/b.m. as in the two figures (4,5), respectively. There were no pathological changes in the histological structure of the liver as the central vein, sinusoids, and hepatocytes were normal, as the results also revealed by examining the histological sections in the sets treated with water seed extract of Moringa oleifera of (450,600) gram/kilogram/b.m. + diclofenac of (100) gram/kilogram/b.m. as in figures (6, 7), respectively.

The set treated with water extract at a concentration of (600) gram/kilogram/b.m. + diclofenac at a concentration of (100) gram/kilogram/b.m. as in figures (8, 9), and the liver tissue was closer to the normal structure with slight changes observed in it such as little necrosis in some liver cells with little widening in sinusoids compared to the control set figure (1).

The results of the study showed the tissue of liver rats treated with diclofenac of (100) gram/kilogram/b.m. had suffered from many pathological changes such as congestion, severe irregularity of the renal cords, and central vein destruction and expansion, and widening into sinusoids, liver cells necrosis and degeneration, and congestion central vein with damage of its wall, necrosis of the liver tissues and, infiltration of inflammatory cells as in figure (2,3) in comparison with the normal histological structure of the livers in the control set figure (1).

The reasons for these results may be attributed to the fact that the water seed extract of Moringa oleifera plant contains materials which had not constitute any toxic impact on the tissues, and did not induce oxidative stress and synthesis of free radicals [18], also, the daily administration the water seed extract of Moringa oleifera ,It has antioxidant and degenerative effects, and this may be due to the various components of the Moringa oleifera seeds, such as vitamins, flavonoids, and phenols, and the water extract of the Moringa oleifera seeds in high doses of body weight did not cause any harm or disease to the liver, this means that giving the Moringa oleifera plant in high doses did not show any clear disease on the liver [19].

This indicates that water seed extract of Moringa oleifera of (450 and 600) gram/kilogram/b.m. is safe and has no side effects, or this may be attributed to ability of seeds extract to increase cellular metabolic processes of liver rat, and that the antioxidant property in Moringa oleifera is due to the presence of phenolic compounds, as the seeds of seed extract of Moringa oleifera contains active compounds such as flavonoids, isothiocins, and glycosides, and these compounds inhibit reactive oxygen species (ROS) [20].

As for the results of the two sets treated with the two different concentrations of the seed extract of Moringa oleifera + diclofenac drug, it may be attributed to this that seed extract of Moringa oleifera works as a
protection against the damage caused by the diclofenac drug because it contains many effective compounds such as flavonoids and phenols, which act on curbing oxidative stress by removing free radicals [21].

The liver histological examinations showed the protective impact of water seed extract of Moringa oleifera in sweeping away free radicals, increasing antioxidant activity, and inhibiting lipid peroxidation, as they are considered effective oxygen species one of the reasons is the hepatotoxicity of the drug [22].

The oral intake of the water extract leads to a reduction in hepatotoxicity and liver damage caused by carbon tetrachloride [23]. While the histological examination of the liver showed damage to the hepatocytes, showing necrotic hepatocytes, infiltration, and numerous inflammatory cells, bloody congestion in the central vein with the destruction of the wall of the central vein, vacuolization of the hepatocyte cytoplasm, widening of the sinusoids, and degeneration of the hepatocytes in the set treated with diclofenac of 100 gram/kilogram /b.m., this may be because diclofenac inhibits the synthesis of prostaglandins, which are considered one of the main components of cells. In the normal state, these components expand blood vessels, and when the drug prevents them, it leads to a lack of access to oxygen and nutrients, and thus there will be destruction, necrosis, and degeneration of tissues and liver cells [24].

Diclofenac and non-steroidal anti-inflammatory drugs, and their consumption in large quantities, lead to toxicity with these drugs [25], and some studies indicated that the toxic hepatic effects of indomethacin led to abnormal changes in the composition of the liver and a difference in the functional parameters of the liver [26]. Histological changes may also be attributed to diclofenac-induced hepatotoxicity due to the reduction of prostaglandin content, which was originally caused by the reduction of the enzyme Cycloogenase (COX-2).

This drug is usually prescribed to relieve pain and fever, has anti-inflammatory activities [27], and excessive use of diclofenac causes many hepatic and renal injuries that have been reported after its administration [28]. Severe hepatocyte injury reflects higher functional liver enzymes, as with the release of these enzymes into the cytoplasm later [29], taking diclofenac of 50 grams/kilogram for 7 days led to an increase in liver enzymes, as this result indicates severe damage [30; 31] and studies have shown diclofenac has a selective impact of inhibition of (COX-1), (COX-2) [32], and (COX-2) It is derived from prostaglandins and has a protective impact towards hepatic harm caused of hepatotoxins like carbon tetrachloride, acetaminophen and others [33].

Figure 1: Histological section of a normal control male liver: A normal central vein (A), hepatocyte (B), sinusoids (C) (H&E Stain 40X).

Figure 2: Histological section of a male rat liver treated with diclofenac (100) gram/kilogram: wall damage of central vein (A), necrosis of the hepatocytes (B),

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enlargement of the sinusoids (C), and necrosis are noted. Hepatic tissue (D) and blood congestion (E) (H&E Stain 40X).

Figure 3: Histological section of a male rat liver treated with diclofenac (100) gram/kilogram: well destruction wall of central vein (A), widening of the sinusoids (B), and necrosis of the hepatic cells (C). (H&E Stain 40X).

Figure 4: Histological section of a male rat liver treated with *Moringa oleifera* seed extract of (450) gram/kilogram, normal of: central vein (A) liver cell (B) Sinusoids. (C) (H & E Stain 40X).

Figure 5: Histological section of a male rat liver treated with *Moringa oleifera* seed extract of (600) gram/kilogram, normal of: central vein (A) liver cell (B) sinusoids. (C) (H & E Stain 40X).

Figure 6: Histological section of a male rat liver treated with *Moringa oleifera* seed extract of (450) gram/kilogram + diclofenac of (100) gram/kilogram: widening of sinusoids (A) necrosis of some hepatic cells (B). (H&E Stain 40X).

Figure 7: Histological section of a male rat liver treated with *Moringa oleifera* seed extract of (450) gram/kilogram + diclofenac of (100) gram/kilogram: a small widening of the sinusoids (A). (H&E Stain 40X).

Figure 8: : Histological section of a male rat liver treated with *Moringa oleifera* seed extract of (600) gram/kilogram + diclofenac of (100) gram/kilogram: a small necrosis in some hepatic cells (A). (H&E Stain 40X).
Figure (9): Histological section of a male rat liver treated with *Moringa oleifera* seed extract of (600) gram/kilogram + diclofenac of (100) gram/kilogram: a small blood congestion is observed in the hepatic vein (A). (H&E Stain 40X).

**Conclusion**

The conclusion from this study is an extract of *Moringa oleifera* seed extract played a protective role by maintaining the histological structure of the liver in male white rats from the adverse and damaging effects caused by the diclofenac drug within the therapeutic dose.

**References**


