Assessment of the hepatoprotective effect of *Moringa oleifera* leaves extract (MOLE) on paracetamol-induced hepatotoxicity in Albino Wistar rats

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https://doi.org/10.59658/jkas.v10i4.1308

Received:
Nov. 8, 2023

Accepted:
Dec. 10, 2023

Published:
Dec. 15, 2023

Abstract

The current study aimed to investigate the hepatoprotective efficacy of *Moringa oleifera* (MOLE) against liver damage in male rats caused by paracetamol. Four groups of twenty-four male Wister rats, weighing between 150 and 200 gm each, were created. To cause liver damage, a dose of 2g/kg of paracetamol was given once a day for a week, and a dose of 300mg/kg B.W. of MOLE was given orally via gavage for four weeks. As an example hepatoprotective medication, silymarin was administered orally at a rate of 100 mg/kg body weight. Enzyme levels in the liver including total bilirubin, ALT, AST, and ALP were utilized to gauge the extent of recovery after hepatic damage. As antioxidant enzymes, SOD and CAT were evaluated. In male rats, paracetamol-induced liver damage was successfully avoided by silymarin and MOLE. The hepatoprotective effects of the extract were evidenced by a noteworthy reduction in the levels of blood enzymes include TB, ALT, AST, and ALP, along with a decline in tumour necrosis factor alpha (TNF-α). Additionally, there was a rise in the levels of antioxidant enzymes SOD and CAT, as well as interleukin 6 (IL-6).

Keywords: *Moringa oleifera* leaves extract, Hepatoprotective activity, Liver injury, Cytokines, liver enzymes.

Introduction

The liver is recognized as a key organ that is involved in many physiological and biochemical processes. These processes include growth, energy production, nutrient supply, and the maintenance of biological systems' regulatory functions. Additionally, the liver is involved in the biotransformation of both endogenous and exogenous substances, such as medications and xenobiotics [1, 2]. Jaundice, fatty liver, and cirrhosis are a few of the ailments that have raised worries about public health all across the world. The main causes of liver damage are hepatotoxic substances including alcohol, drugs, and viral infections [1, 3]. More over 2 million people die each year from liver disease, even though cirrhosis affects 4.5 to 9.5% of persons around the world, 18.5%

of people have chronic liver disease [4]. The potential for serious side effects from synthetic or traditional medications may be the reason for this [5]. As a result, a wide range of medicinal herbs have been investigated for their potential hepatoprotective and regeneration effects [3]. According to reports, the components of these 101 plants —that is, more than 160 phyto-chemicals—were responsible for the potential hepato-protective activity [6]. These medicinal plants are regarded as an essential source of medication for liver conditions like cirrhosis, hepatitis, and appetite loss [7].

Many nations, especially India, cultivate *moringa oleifera*, which has long been utilized in traditional herbal therapy [8]. It is well known as miracle tree due to their constituents such as micronutrients including β-carotene, A variety of minerals, including calcium, potassium, iron, isothiocyanates, and polyphenols, as well as vitamins C, K, E, D, B1, B2, B3, B6, and B12 [9]. Furthermore, ellagic acid, apigenin, quercetin, and kaempferol are the most antioxidants constituents [10] that contribute to its hepatoprotective and antioxidant effects in rats [11]. Several MO extracts have been shown to be relatively safe; one such extract has an alcoholic leaf median lethal dose (LD50) of up to 5 gm/kg body weight (BW) [12]. Studies conducted on humans, animals, and plants in vivo as well as in vitro have demonstrated that different MO leaf extracts have distinct medicinal effects, including chemoprotective (hepatic, cardiac, renal, and nervous system) actions, antioxidant properties, anti-inflammatory, and hypolipemic effects [13, 14]. The development and progression of the inflammatory response to different pollutant exposures are significantly influenced by oxidative stress; for this reason, the reciprocal relationship between oxidative injury and inflammatory processes was taken into consideration [15]. All stages of the inflammatory process are amplified by oxidants, including the generation of cytokines that promote inflammation, the signaling pathways' activation, and the adaptive cell response [16].

The non-steroidal anti-inflammatory medication (NSAID) such as paracetamol is well known. Owing to its accessibility and the belief that it is reasonably safe when taken as prescribed, it can cause significant liver damage in long-term abusers [17]. The purpose of the current study was to investigate the hepatoprotective effects of extract from Moringa oleifera leaves in rats that were exposed to toxicity of paracetamol.

Materials and Methods

Preparation of plant material

After being thoroughly cleaned with distilled water, the plant leaves were left to dry for 21 days at room temperature. A high-speed milling machine was used to grind the dried MO leaves into a fine powder. Subsequently, two times, 1000 mg of the powder was extracted in 1000 mL of absolute ethanol and filtered through filter paper with pores measuring 2 µm. A rotary evaporator was used to evaporate the resulting ethnolic extract at a temperature of 50 °C. For every 1000 grams of dried powder, the obtained MOEE extract had a residual yield of 78.3 grams. After the extract was obtained, it was reconstituted in a brown bottle (1 g of extract: 10 mL of distilled water) and kept cold until it was needed.

Animals

Wister albino rats, male, 150–200 g, were acquired from the University of Karbala's College of Veterinary Medicine's animal house. Standard laboratory settings were applied to the animals, who were housed in cages with 12 hour cycles of day and night and 25–28 °C air conditioning. The experimental treatments were started after the rats had had a week to get used to the lab environment. Rats were fed pellets regularly and provided unlimited access to water.

Chemicals

The materials used in this experiment were exclusively analytical in nature. Paracetamol and silymarin (E. Merck) (Sigma Chemical Co.). Bio Lab produced diagnostic kits to measure serum transaminases (ALT, AST), alkaline phosphatase (ALP) enzymes, serum bilirubin, and antioxidant enzymes like catalase and superoxide dismutase. In addition, ELISA kits (Assaypro LLC, Charles, MO, USA for TNF-α and Sigma Chemical Company, USA for IL-10) were used to estimate IL-10.

Experimental Design

Following adaption, twenty-four animals were randomly separated into four groups of six: First Group: these rats were the control group and were given distilled water. Rats in the second group were given an amount of 2 g/kg of paracetamol once a day for a week in order to cause liver damage. Rats in the third group were given 300 mg/kg B.W. of MOLE orally via gavage for a period of four weeks after the paracetamol administration. The fourth group followed by administering silymarin orally as a standard liver-protective medication at a rate of 100 mg/kg B.W. after paracetamol was administered.

Biochemical, antioxidant enzyme and cytokines parameters

Before being sacrificed, the animals were fasted for a full night, using anesthesia drugs like ketamine and xylazine, followed by cervical dislocation. Blood was then drawn from each group to measure the levels of serum ALT, AST, ALP, and TP as well as antioxidant parameters like SOD and CAT using commercial kits (Bio Lab). TNF-α and IL-10 were estimated by ELISA kit.

Histological assessment

Following the animals' rapid sacrifice, the livers were extracted, and minute pieces of each liver were kept in a 10% formalin solution before being subjected to industry-standard micro processing techniques for paraffin embedding. Hematoxylin-eosin (H&E) was used to stain liver tissue sections that were 5 µm thick such that the changes in histopathology could be seen under a microscope with a light source [18].

Statistical Analysis

The results of the analysis of all the data were displayed as mean standard error of the average using the GraphPad Prism software version 10 for Windows. A comparative statistical analysis was conducted prior to performing normality tests utilizing the Shapiro normality test as well as analysis of variance (ANOVA) with a numerous comparison test of Turkey. $P \leq 0.05$ was used to determine significance.

Results and Discussion

Biochemical parameters

Studies were carried out to evaluate a few biochemical parameters related to liver damage caused by paracetamol in male rats. The results showed that the paracetamol-treated group had significantly higher levels of ALT, AST, ALP, and TP than the control group ($P < 0.0001$). When compared to the paracetamol group, MOLE and Silymarin dramatically reduced the levels of AST, ALT, ALP, and TP ($P < 0.0001$) (Figure 1). Additionally, after taking paracetamol, there was no significant difference in AST, ALT, ALP, and TP between the Silymarin-treated group and MOLE (Figure 2).

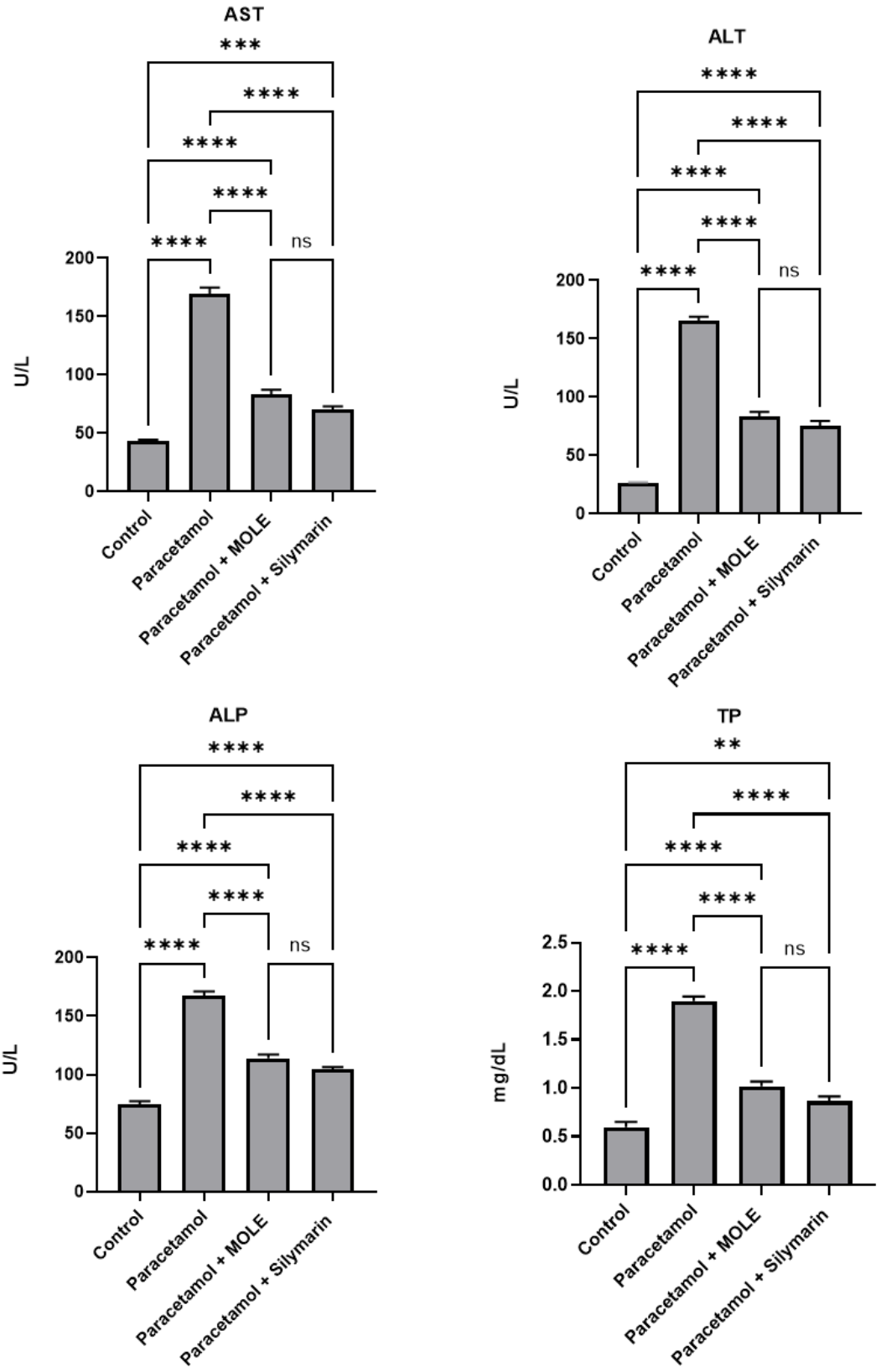

Figure (1): Effect of MOLE on serum biochemical levels following treated groups in male rats Data are expressed as mean±SEM (n=6).

Antioxidant parameters

Experiments were carried out on male rats to evaluate certain antioxidant parameters in liver damage caused by paracetamol. The results indicated that the paracetamol-treated group had significantly lower levels of both CAT and SOD than the control group ($P < 0.0001$). Figure 3 shows that both CAT and SOD levels were significantly higher in the MOLE and Silymarin group than in the paracetamol group ($P < 0.0001$). Furthermore, there was no difference in SOD levels observed between the silymarin-treated and MOLE-treated groups (Figure 2).

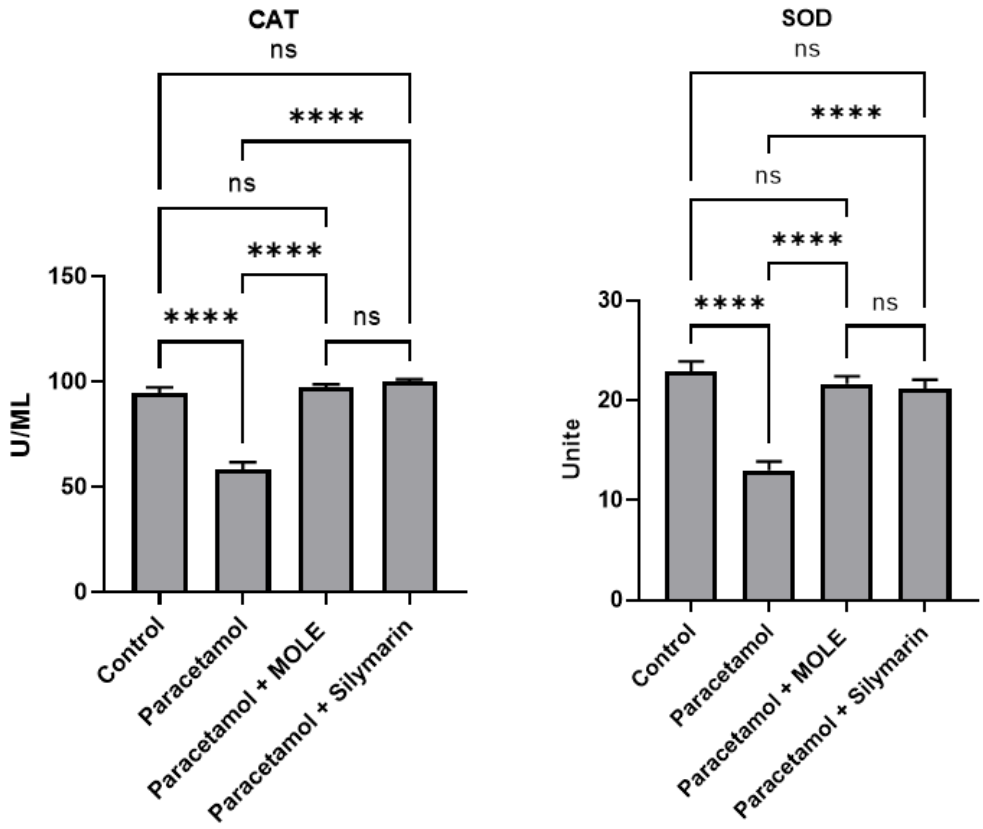

Figure (2): Effect of MOLE on serum antioxidant enzyme levels following treated male rats. Data are expressed as mean±SEM (n=6).

Measurement of the levels of TNF-α and IL-10

Experiments were carried out on male rats to evaluate certain cytokines i.e. TNF-α (inflammatory mediator) and IL-10 (anti-inflammatory mediator) in liver damage caused by paracetamol. Figure 3 shows the effects of paracetamol and/or MOLE on pro- inflammatory mediator i.e. TNF-α and anti-inflammatory i.e. IL-10 levels in the male rats. Administration of paracetamol significantly ($P < 0.0001$) increased the levels of TNF-α while it significantly ($P < 0.0001$) decreased IL-10 (Figure 4 C) in treated

animals compared to the control group. However, administration of MOLE to paracetamol-intoxicated rats significantly ($P < 0.0001$) decreased the TNF-α levels (Figure 4) while significantly ($P < 0.0001$) increased IL-10 content. Additionally, after taking paracetamol, there was a significant difference in TNF-α and IL-10 between the Silymarin-treated group and MOLE (Figure 3).

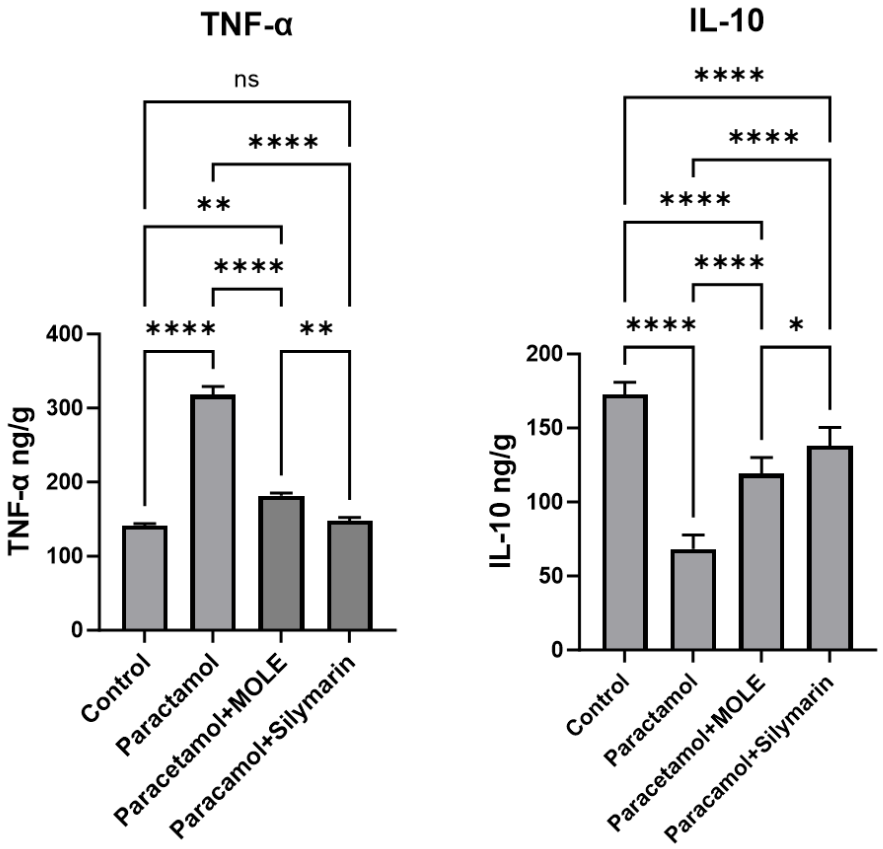

Figure (3): Effect of MOLE on serum cytokines levels following treated male rats Data are expressed as mean±SEM (n=6)

Histopathological studies of liver section

Figure 4 (A) photomicrograph of the liver of control group animal, showed normal hepatic structure manifested by the normal hepatocytes cords (black arrow) around the central vein (red arrow) and normal sinusoids (white arrow) While, figure 5 (B) photomicrograph of liver of paracetamol-induced liver injury group, showed sever central vein congestion (black arrow), sever sinusoidal dilatation (red arrow) with significant degeneration of hepatocytes (white arrow). In addition, the histopathological changes in the liver section of liver of paracetamol induced liver injury group, revealed wide area of lobular hepatocytes necrosis (black arrow), sever exudation in blood vessels (red arrow) with significant depletion of hepatocytes (white arrow) figure 5 (C). Bile

ducts proliferation in portal area (black arrow), moderate to severe hepatocytes fatty change (red arrow) with necrotic hepatocytes (white arrow) can be seen the liver from the paracetamol-induced liver injury group figure 5 (D). Furthermore, extensive fibrotic bundles proliferation around portal vein (black arrow), sever inflammatory cells infiltration (white arrow) figure 5 (E). Interestingly, sections of liver from the treated group, revealed mild necrosis of hepatocytes (black arrow), marked degenerative changes of hepatocytes (white arrow) figure 5 (F) as well as mild fibrosis around and in portal area (black arrow),marked portal vein congestion with exudation (white arrow) and mild inflammatory cells infiltration (yellow arrow) figure 5 (G).

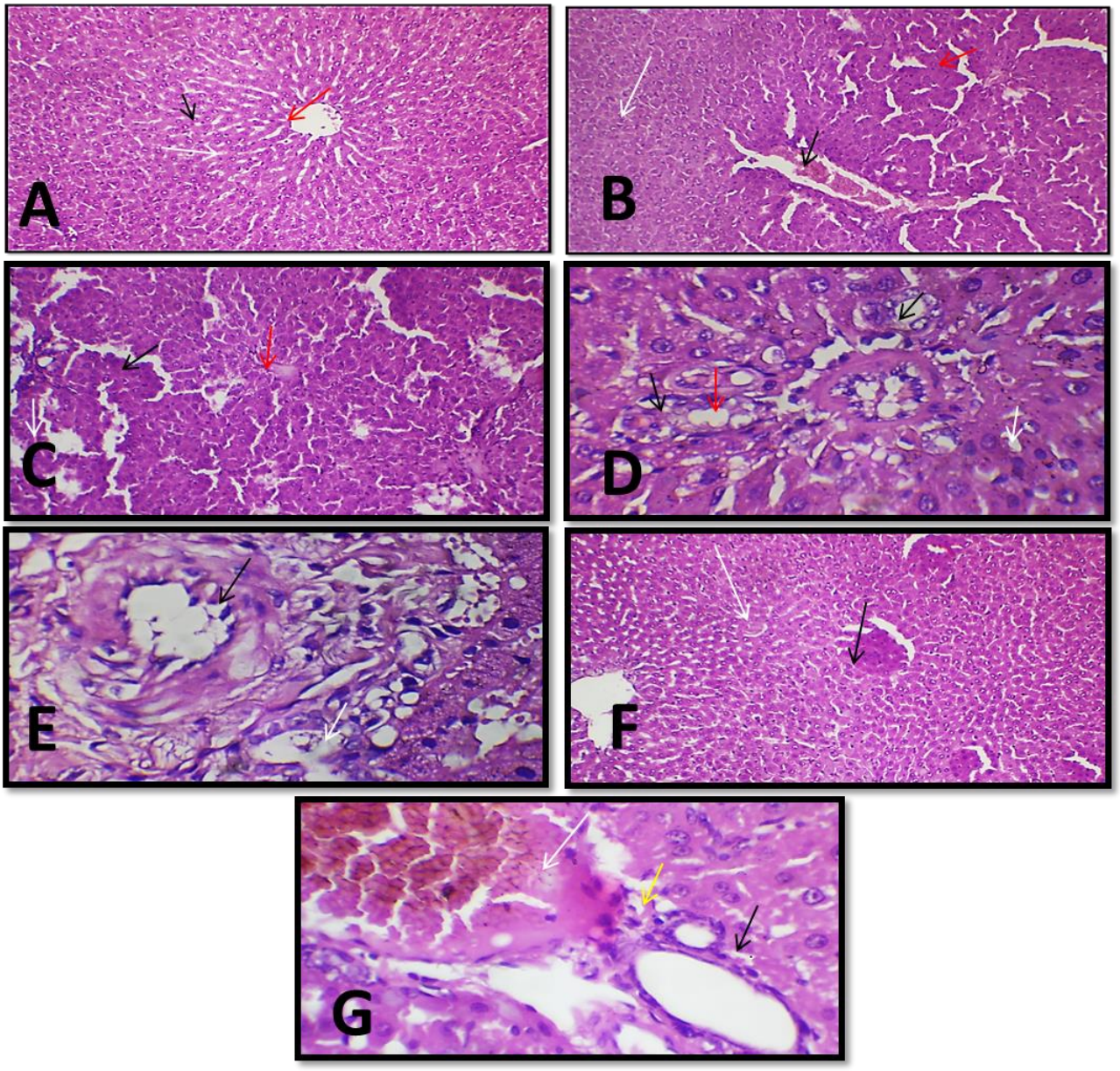

Figure (4): Histopathological effects of the liver sections

This study's experiments observed at the hepatoprotective benefits of extract from Moringa oleifera leaves (MOLE) in rats that were exposed to paracetamol toxicity. In order to cause liver toxicity, 2g/kg B. Wt. of paracetamol was used. The information provided here demonstrates that consuming this amount of paracetamol can cause significant liver damage. This was observed in the histopathological alterations for the liver sections stained with the common histopathological stain, hematoxylin and eosin stain, as well as the data gathered from several biochemical and antioxidant parameters. The administration of 300 mg/kg B.W.T. of MOLE showed hepatoprotective and antioxidant action against paracetamol-induced liver damage, which was an important finding. This could be explained by MOLE's phytochemical screening.

In the current investigation, paracetamol intoxication was followed by a rise in the serum enzyme activity of ALT, AST, and ALP. This outcome could be explained by changes in the plasma membrane of liver cells brought about by oxidative stress, which can also contribute to the release of cytosolic enzymes such as ALT, AST, and ALP and raise serum levels at the same time [17]. Consequently, measuring the activity of these enzymes in the blood is useful in the study of liver function [17]. On the other hand, the current investigation found that administering Moringa oleifera raises serum levels of total protein, AST, ALP, and ALT significantly.improvement following a paracetamol overdose. The administration of the leaves of Moringa oleifera extract has been shown to have an anti-hepatotoxic effect, as seen by the reduction in ALT, AST, and ALP activities. This improvement in hepatic cell cellular membrane integrity is likely the result of this action occurring early. These results corroborated those of [20] who revealed that moringa lowers lipid MDA, a peroxidation biomarker, while raising the activity of the antioxidant enzymes, namely SOD, CAT, and GPX.

The results of the study demonstrated that the groups varied significantly during the experiment. For example, the antioxidant enzymes, like SOD and CAT, were significantly reduced in the animals that were exposed to 2g/kg paracetamol, suggesting that the drug significantly decreased the hepatic antioxidant status. In contrast, MOLE increased the antioxidant activity of the enzymes (figure 3). This might be attributed to MOLE's phytochemical screening uncovers antioxidant phytochemicals such as triterpenes, phenols, sterols, flavonoids, and saponins, which may be involved in the plant's hepatoprotective properties. MOLE demonstrated antioxidant activity comparable to that of common antioxidants such as gallic acid, as per a study by [8]. Total extracts from all of MOLE's parts also showed potential antioxidant activity, matching that of ascorbic acid, the industry standard antioxidant, according to [9, 20].

The inflammatory response being activated by paracetamol can be explained by its toxic action, as seen by a decrease in IL-10 levels and a rise in TNF-α in serum blood samples (figure 4). On the contrast, administering of Moringa oleifera raises serum levels of anti-inflammatory mediator IL10 while the pro inflammatory mediator i.e. TNF-α has been decreased. This is due to the phytochemical constituents of the plant extract. In support with these finding, [19] had reported that MOLE can exert hepatoprotective effects.

The results of this study demonstrated that MOLE was unquestionably protective against the harmful effects of paracetamol on the livers of the rats. Histological findings and hepatotoxicity indicators show that the protective efficacy of MOLE at a dose of 300 mg/kg/day is very similar to that of silymarin at a dose of 100 mg/kg by increasing both the antioxidant enzymes and anti-inflammatory mediator and decreasing pro-inflammatory mediator.

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