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Investigation of the Hepatoprotective, Antoxidant and Antifibrotic Effects of Desert Date (*Balanites aegyptiaca*) Oil in an Experimental Model of Liver Fibrosis in Rats

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ABSTRACT

Background: Hepatic fibrosis is one of the most common lesions during chronic hepatic diseases. It is a significant health issue in many countries because it can lead to cirrhosis and ultimately liver failure without careful treatment. No successful management to treat this condition has been established so far. Some medicinal plants are particularly important in this sense because of the intrinsic healing ability of their plants, as well as their long-term therapeutic capabilities and few side effects. The hepatoprotective role of desert date oil (DDO) is clearly indicated in recent studies; however, the accurate mechanisms are still unknown. **Objectives**: The present study was designed to investigate the hepatoprotective, antioxidant, and antifibrotic effects of desert date oil against the hepatic damage and oxidative stress induced by thioacetamide (TAA) administration in male rats. **Material & Methods**: In this study, 40 healthy adult albino rats were divided into four groups: group 1 (control), group 2 received orally DDO (500 mg/kg b.wt/orally every day), group 3 was injected intraperitoneally by TAA by intraperitoneal (I.P.) injection of 200 mg/kg b.wt, twice weekly to induce liver fibrosis, group 4 was injected by I.P. TAA and received DDO orally every day by the same doses as above. At the end of the experiment (8 weeks), the rats of different groups were sacrificed, and blood samples were collected for the determination of the liver function tests. Then, the abdomen of rats of different groups was opened, where the liver was removed, weighted and cut into small pieces; some pieces were homogenized to measure the oxidative (malondialdehyde [MDA]) (MDA) and antioxidative parameters (superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), while other pieces were processed for different histological and immunohistochemical techniques. **Results**: The present study revealed that TAA administration to rats resulted in a significant decrease in body weight gain with an increased liver/body weight ratio (liver weight index). In addition, the levels of liver enzymes (ALT, AST, and ALP) and total bilirubin were significantly increased, while the levels of total protein and albumin were statistically decreased. TAA led to a significant increase in MDA, a lipid peroxidation marker, with a concomitant decrease in antioxidant enzymes (SOD, CAT, and GPx) when compared with the control group. The histopathological examination showed abnormal changes characterized by an obvious increase in the collagen content, and bridging fibrosis with the hepatic cells showed various degenerative changes in the cytoplasm and nucleus. The administration of DDO along with TAA resulted in improvements in all biochemical tests and histopathological changes and significantly attenuated TAA-induced oxidative damage. **Conclusion**: This study revealed that desert date oil has antifibrotic and hepatoprotective effects against the liver fibrosis, biochemical and structural changes induced by TAA administration in rats; possibly due to its antioxidant properties. Additionally, the obtained results

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confirmed that this oil is a potential protective natural therapy against liver fibrosis induced in the course of many chronic liver diseases.

Keywords: Hepatic fibrosis, chronic hepatic diseases, hepatoprotective, antioxidant, antifibrotic

1. Introduction

Liver fibrosis becomes one of the most frequent lesions in chronic hepatic diseases, defining it as a wounding reaction to various kinds of injuries that is characterised by an increased aggregation in various sections of hepatic lobules of fibrous or extracellular matrix protein components (e.g., collagens), which disturbs the natural architecture of the liver (Berumen *et al.*, 2020). In developed nations, the most prevalent causes of liver fibrosis are alcohol abuse, viral hepatitis B and C diseases, and metabolic syndromes associated with obesity, glucose intolerance, and diabetes (Carvajal *et al.*, 2019; Lin *et al.*, 2019). Parasitic diseases, such as *Schistosoma* parasites, are mostly demonstrated in the case of liver damage in non-industrialized countries (Pinzani, 2015; Bergquist *et al.*, 2018; McCarthy *et al.*, 2021).

Formation of liver fibrosis progressively develops across many years without proper care or even without regulation of the fibrosis, leading lobular architecture to be distorted, triggering nodular formation, permanent cirrhosis, portal hypertension, and liver damage or bleeding from esophageal varicose veins (Friedman, 2008; Kawada, 2011; Park *et al*., 2021; Roccarina *et al.*, 2021). Moreover, in general, the risk of liver cancer has been shown to be increased. Cirrhosis as it takes hold, should be taken as a symptom of liver cancer (Fujiwara *et al*., 2018; Dhanaraj *et al*., 2020).

Indeed, liver fibrosis is a widely held and complicated clinical issue that is important worldwide and is regarded as the principal causes of morbidity and mortality, with a significant economic burden on the population, in particular in developing nations with therapeutic limitations (Al-Attar and Al-Rethea, 2017; Bassetti *et al*., 2017; Önnerhag *et al*., 2019). Hepatic disorders have developed internationally in recent decades. A 2016 study conducted by the Centers for Disease Control and Prevention (CDC) revealed the impact of 4.9 million people. The increased incidence of nonalcoholic steatohepatitis and hepatitis viruses is likely to raise this burden in the coming months (CDC, 2016; 2017; Roccarina *et al*., 2021; Younossiet *et al*., 2021).

Liver fibrosis is currently a complex and bilateral method that includes the imbalance between the formations of the different components in connective tissue (Friedman, 2010; Scholten *et al*., 2011; Berumen, 2020). This opinion has been validated by experimental animals and clinical studies that show that liver fibrosis is a dynamic process with a potential for reversibility after stopping causative agents or eliminating triggers of chronic or recurrent inflammatory in the liver (Iredale *et al*., 2017; Tsuchida and Friedman, 2017; Cholankeril and Ahmed, 2018; Muntean, 2020).

In the recent period, in initiating molecular events that are responsible for this fibrotic process, the underlying mechanism of liver fibroids was identified as involving the development and significant decrease of oxidative pressure (ROS) and antioxidant status (George *et al*., 2019; Fu *et al*., 2020; Li *et al*., 2021). Up untill now, no efficient therapy for this liver disease has yet been established. The latest synthetic therapies for chronic hepatic disease are not appropriate (Saha *et al*., 2019; Nag *et al*., 2020; Pydyn *et al*., 2020). While considerable progress has been achieved, including chemical, medicinal, and surgical therapy, none of the solutions is sufficient or hindering progress on hepatic disease as well as their major side effects, to combat or remove inflammatory triggers that influence their growth. Therefore, modern and secure alternate methods for either liver disease protection or treatment that contribute to fibrosis are definitely necessary (Nouri-Vaskeh, 2020; Al-Qahtani, and Binobead, 2019; Kalra *et al*., 2019).

In light of this, certain medicinal plants earn special consideration owing to their well-known natural healing process, long-term curative properties and their insignificant side effects. Natural biological compounds, particularly polyphenols, are potential agents for the prevention and treatment of liver diseases (Cao *et al*., 2020). Moreover, some farming and medicinal herbal oils are considered a nutrient and fat-soluble source of vitamins (Macho-González *et al*., 2020; Orabi *et al*., 2020).

Balanites aegyptiac seed oil has significant medical benefits in many countries in the Middle East. *Balanites aegyptiaca* was a universal public accepted folk medicine (Patil *et al*., 2019; Dehyab *et al*., 2020), which was used for the treatment of various ailments such as jaundice, liver problems,

epilepsy, and yellow fever (Khanam and Galadima, 2021). Many studies have clearly suggested the hepatoprotective effect of *Balanites aegyptiaca* oil; however, the exact mechanisms remain unclear (Ali *et al*., 2019; Ezemokwe *et al*., 2020). The interactive effect of *Balanites aegyptiaca* oil was found to reduce the liver injury induced by TAA admission, liver inflammation, tumor progression and other clinical problems (Mishra *et al*., 2014).

Balanites aegyptiaca 'desert date' is a socio-economic priority forestry plant for the rural population. The dietary intake of this fruit is especially useful in terms of nutrition for locals (Sambo *et al*., 2020). It is a spiny shrub or tree that is widely distributed in drylands in Africa and South Asia up to a height of 10 meters. This is typically used for the diagnosis of specific diseases, including jaundice, bowel worm infection, fractures, malaria, hysteria, insanity, yellow fever, and syphilis (Yadav and Panghal, 2010). It contains protein, lipid, carbohydrate, alkaloid, saponin, flavonoid, and organic acid (Chothani and Vaghasiya, 2011).

The presence of various phytochemicals such as cardiac glycosides, flavornoids, and polyphenols has established some biochemical basis for ethno-pharmacological uses of *Balanites aegyptiaca* components to treat and prevent various diseases and disorders (Tula *et al*., 2014). The mesocarp of *Balanites aegyptiaca* fruit basically contains 1.2 to 1.5% proteins, 35 to 37% sugars, and 15% organic acids. Chemical analysis of *Balanites aegyptiaca* revealed other compounds such as 3-rutinoside, 3-rhamnogalactoside, and diosgenin (Chothani Vaghasiya, 2011). This study was designed to investigate the protective effect of desert date oil on thioacetamide-induced liver injury in rats

2. Material and Methods

2.1. Ethical approval

All the animal protocols and procedures used in this study were approved by the Committee of Animal Investigations, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia.

2.2. Drugs and chemicals

TAA of high analytical grade and all other chemicals, stains and kits were purchased from Sigma-Aldrich group, St. Louis, MO, USA *via* specified scientific agents, Jeddah, Saudi Arabia. TAA solution was prepared by melting in 10% Tween 20 and mixing well until complete dissolved (Amin *et al*., 2012).

2.3. Preparation of desert date oil (DDO):

The fruits of *Balanites aegyptiaca* were obtained from the University Farm project in Hada Alsham, 24 km from Jeddah city center, Faculty of Meteorology, Environment and Arid Land Agriculture. The fruits were botanically identified as parent plant in the Department of Phytochemistry at the Faculty of Pharmacy, king Abdulaziz University. The fruits were properly washed in water, and rinsed using distilled water. The outer cover (epicarp) was removed by using a sharp blade to acquire the fruits. Pre-processing consists of the elimination (de-hulling) of *Balanites aegyptiaca.* The pre-processing that includes soaking fruits in water for three days and scraping the pulp helps us to acquire fruit. Fruit were dried by solar means. Seeds could be harvested by smashing fruit with a heavy, sturdy object, such as a hammer, on a hard, flat surface (Bambara *et al.*, 2018). The method of extraction requires somehow heating the crude. Oil extraction, seems does not sustain the major loss at the low temperatures (12-11 °C to 70 °C) but decreased their yield (Fawad, 1933).

2.4. Animal groups and experimental work

Forty male *Wistar* rats (180–220 gm) were obtained from the Experimental Animal Unit of King Fahd Medical Research Center (KFMRC), King Abdulaziz University, Jeddah, Saudi Arabia. The rats were acclimatized to the laboratory conditions for one week prior to the commencement of the experiments where they were housed in well-aerated standard plastic cages and maintained under controlled laboratory conditions of humidity (55% ±10), constant room temperature (20 ± 2 °C) and 12:12 h light: dark cycle each day. Rats were fed with standard laboratory chow diet ad libitum with free access to water. Before the start of the experiment, the rats were divided into seven groups (n=10 for each group):

G1 (Negative control group): The rats were given 2 ml of distilled water by oral gavage in the same times of administration of drugs to the other groups.
G2 (DDO-treated group): The rats were given 500 mg kg b.wt. / day of DDO by oral gavage.
G3 (TAA group): The rats received intraperitoneal injection of TAA in a dose of 200 mg /kg body weight (b.wt.) by intraperitoneal injection (I.P), twice weekly to induce liver fibrosis (Aydin *et al*., 2010; Amin *et al*., 2012). These rats were kept as positive control group.
G4 (TAA + DDO group): The rats were injected with I.P TAA at the same way as the previous group and given 500 mg kg b.wt./ day of DDO by oral gavage.

Throughout the experiment period (8 weeks) and during all the steps of the study, the animals were observed daily for health status and signs of abnormalities. The body weight and food intake were monitored regularly. At the end of the experimental period, rats were fasted over night; and then anaesthetized with diethyl ether. Blood samples were collected from retro-orbital venous plexus using non-heparinised tubes, and then centrifuged at 3000 rpm for 15 minutes for serum separation. The blood sera were then collected, frozen at -80 °C and stored for further biochemical analysis. Next, each rat's abdominal cavity was opened where the entire liver was removed, washed with ice cold saline then plotted with filter paper; then weighted for determination of liver weight. Then, mall pieces (1/2 cm^2) from the right lobe of liver were taken and fixed in 10% buffered neutral formalin solution (NBF) for histopathological and immunohistochemical studies. In addition, other liver pieces were frozen in liquid nitrogen and stored at -70 °C for the determination of oxidant and antioxidant markers.

2.5. Assessment of body weight gain, liver weight and food intake values

The body weight of the rats was recorded at start (IBW) and at end (FBW) of the experimental duration of different groups using a digital balance. The body weight gain (BWG) was calculated according to the following equation:
Body weight gain (BWG) = Initial body weight (g) - Final body weight (g) - Initial body weight (g).
Also, the relative liver weight was calculated according to the following equation:

$$\text{Ratio} = \frac{\text{Liver weight}}{\text{Body weight}} \times 100$$

The food intake value was determined every other day by reducing the weight of the remind chow pellets from the weight of initial amount as follows:

Food intake (FI) = Initial amount weight (g) - Remained amount weight (g)

2.6. Assessment of liver function tests

Using an Olympus AU-2700 autoanalyzer (Olympus, Hamburg, Germany) and market kits, the levels of the following parameters in the serum samples from the rats of different groups were assessed spectrophotometrically according to manufacture information. These include liver enzymes: aspartate amino transaminase (AST), alanine amino transaminase (ALT), alkaline phosphatase (ALP), total bilirubin (TB), total protein (TP) and serum albumin.

2.7. Preparation of tissue homogenates and measurement of oxidative parameters

The frozen liver pieces from the rats of different groups were left to defrost and mixed with 2 ml ice-cold Tris–HCl, pH 7.4 and containing 1% protease inhibitor. The mixtures were homogenized at a speed of 16,000 rpm, and then buffer was added until the final volume was ten times the weight of the tissue. The supernatants were collected and used for assessment of the levels of lipid peroxidation marker and antioxidative enzyme activities. This was done by using a spectrophotometer (Pharmacia Biotech., Cambridge, England) by means of kits provided by Biodiagnostic Co, USA as follows: the lipid peroxidation product was measured by malondialdehyde (MDA) according to the method used by (Mihara and Uchiyama, 1978) and the tissue MDA content was expressed as nmol/g tissue. Superoxide dismutase (SOD) activity was assessed according to (Sun *et al*., 1988) and its activity was expressed in U/g tissue. Catalase (CAT) activity was assessed according to (Aebi *et al.*,

1984) and its activity was expressed in U/g tissue. Glutathione peroxidase (GPx) activity was assessed as by (Koracevic *et al*., 2001) and it is activity was expressed in mU/g tissue.

2.8. Histological study

The NBF-fixed liver pieces were processed through up-graded alcohol (60%, 70%, 80%, 90% and 100%) and xylene and then embedded in paraffin blocks using an automatic processor. The blocks were cut into 5 um sections, which were routinely stained with haematoxylin and eosin (H&E) to assess the liver architecture and with Masson's Trichrome (MT) to examine the fibrous tissue content (Bancroft and Layton, 2013).

2.9. Immunohistochemical methods

For immunohistochemical staining, other liver sections were processed using streptavidin biotin-peroxidase method according to (Horiguchi *et al*., 2007). The slides were deparaffinized, rehydrated and treated with 0.01 M citrate buffer (pH 6.0) and kept in microwave oven at 500W for 10 min for antigenic retrieval. Then, the slides were washed with phosphate buffer solution (PBS) for 5 min followed by incubation over night at 4°C in a humidified chamber with the following primary antibodies: polyclonal rabbit anti-caspase 3 at 1:50 dilution (Cell Signalling Technology Inc., MA, USA) at dilution of1:100, mouse-monoclonal Proliferating Cell Nuclear Antigen (PCNA) antibody at 1:100 dilution (clone PC10, DAKO Corp. Denmark). The immunoreaction was detected using peroxidase-labeled biotin–streptavidin, supplied in the Super Sensitive TM MultiLink-Label ICH Detection System (BioGenex, San Ramon, CA) and visualized with the chromogen 3,30-diaminobenzidine tetrahydrochloride (DAB) (Bancroft and Layton, 2013). Finally, the sections were counterstained with hematoxylin and left to dry and then mounted in DPX mount and covered by the cover slip. The histography of the prepared slides from different groups was done using an Olympus BX53 microscope equipped with a camera (Olympus, Tokyo, Japan) at different magnifications.

2.10. Statistical analysis

Data were presented as mean +/- standard deviation (SD) and were analyzed by IBM SPSS Statistics for Windows, version 23 (IBM SPSS, IBM Corp., Armonk, N.Y., USA). Shapiro – Wilk test was used to evaluate normal data distribution. Statistical comparisons between groups made by One-Way analysis of variance (ANOVA) followed by least significant test (LSD). Results considered statistically significant if P-values were <0.05.

3. Results

3.1. General observations

In the TAA-treated rats, loss of appetite, decreased activity, weakness, and yellowish body hair were observed during the experimental period. In addition, two mortality cases were recorded at 4^{th} and another three at 5^{th} weeks. On the other hand, the rats in the other groups appeared healthy and did not show clinical signs of toxicity and no mortality was recorded during the experimental duration.

3.2. Effects on body weight gain, food intake and liver weight

As seen in Table 1 and Figure 1, the results of body weight gain, liver weight, and food intake were more or less similar in the control and DDO-treated groups without any significant differences. There was a significant reduction in body weight gain and food intake in the TAA-treated group ($P<0.0001$), when compared with the control group. Meanwhile, administration of DDO to the TAA-treated group resulted in an improvement in both parameters compared to the control figures ($P<0001$). Moreover, there were insignificant changes in absolute liver weight between the different studied groups ($P>0.05$). However, a significant increase in the relative liver weight ratio was observed in the TAA-treated group ($P<0.0001$) as compared to the control group, whereas co-treatment of DDO with TAA resulted in its decrease towards normal.

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Table 1: Effect of TAA and DDO treatments on body weight gain, liver weight and food intake (gm) in different experimental groups

Groups	Body Wt. Gain (BWG)	Food Intake (FI)	Absolute Liver Wt. (ALW)	Relative Liver Wt. Ratio (RLWR)
Control group (G1)	131.9 ± 11.3	38.1 ± 3.7	8.33 ± 1.77	6.32 ± 0.88
DDO-treated group (G2)	128.4 ± 9.7	35.3 ± 2.9	7.86 ± 1.92	6.18 ± 1.04
Significance	2P <0.0001***	2P <0.0001***	2P =0.1458	2P <0.0001***
TAA-treated group (G3)	92.5 ± 7.3	22.4 ± 2.8	9.18 ± 1.48	9.82 ± 0.74
Significance	1P <0.0001***	1P <0.0001***	1P =0.3151	1P <0.0001***
TAA + DDO-treated group (G4)	118.7 ± 9.3	33.7 ± 4.1	8.78 ± 1.38	7.39 ± 0.68
Significance	1P =0.0106*, 2P <0.0001***	1P =0.0408*, 2P <0.0001***	1P =0.5796, 2P =0.5849	1P =0.0166*, 2P <0.0001***

Data are expressed as mean ± SD, 1P: significance versus G1; 2P: significance versus G4. *: P <0.05, **: P <0.010, ***: P <0.0001. Significance was made using OneWay ANOVA test followed by Turkey test.

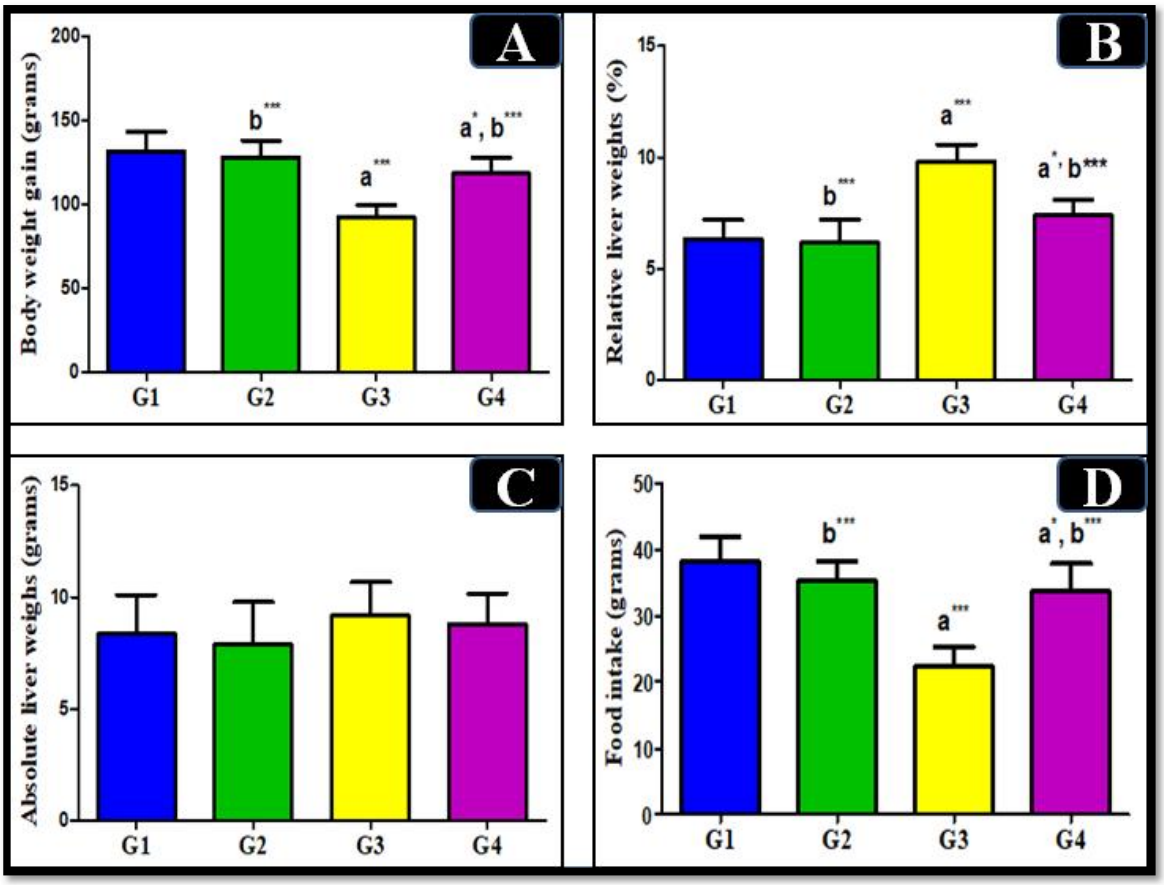


Fig. 1: Columnr charts showing the effect of TAA and DDO on (A) body weight gain (grams), (B) body food intake (grams), (C) absolute liver weight (grams) (D) relative liver weight (%) in different experimental groups. a: significance versus G1; b: significance versus G4.*: P <0.05, **: P <0.010, ***: P <0.0001. Significance was made using OneWay ANOVA test followed by Turkey test.

3.3. Liver functions parameters

The data in Tables 2 & 3 and Figures 2 & 3 revealed that in TAA-treated rats, there was a significant elevation (P<0.0001) of mean values of liver enzymes; (ALT, AST and ALP) and total bilirubin in comparison to control. In contrast, the mean values of total protein and albumin were significantly decreased (P<0.0001). However, the co-treatment TAA with DDO to rats showed improvement of all these parameters, which were comparable to control values.

Table 2: Effect of TAA and DDO treatments on liver enzymes in different experimental groups

Groups	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
Control group (G1)	28.8 ± 3.14	25. 9 ± 3.33	78.4 ± 7.22
DDO-treated group (G2)	32.4 ± 2.08	29. 3 ± 3.39	71.7 ± 5.14
Significance	2P <0.0001***	2P <0.0001***	2P <0.0001***
TAA-treated group (G3)	118.8 ± 7.84	96.6 ± 6.48	152.2 ± 16.11
Significance	1P <0.0001***	1P <0.0001***	1P <0.0001***
TAA + DDO-treated group (G4)	38.2 ± 5.19	37.6 ± 4.04	89.4 ± 7.74
Significance	1P =0.0006***, 2P <0.0001***	1P <0.0001***, 2P <0.0001***	1P =0.0108**, 2P <0.0001***

Data are expressed as mean ± SD, 1P: significance versus G1; 2P: significance versus G4. *: P <0.05, **: P <0.010, ***: P <0.0001. Significance was made using OneWay ANOVA test followed by Turkey test.

Table 3: Effect of TAA and DDO treatments on total proteins, albumin and total bilirubin in different experimental groups

Groups	Total proteins (g/dl)	Albumin (g/dl)	Total bilirubin (mg/dl)
Control group (G1)	7.55 ± 1.30	4.81 ± 0.91	1.10 ± 0.03
DDO-treated group (G2)	7.48 ± 1.08	4.72 ± 0.96	1.19 ± 0.07
Significance	2P <0.0003***	2P <0.0002***	2P <0.0001***
TAA-treated group (G3)	5.08 ± 0. 92	2.75 ± 0.55	2.82 ± 0.08
Significance	1P <0.0006***	1P <0.0001***	1P <0.0001***
TAA + DDO-treated group (G4)	7.12 ± 1.16	4.58 ± 0.92	1.32 ± 0.14
Significance	1P =0.4966, 2P =0.0016**	1P =0.623, 2P <0.0003***	1P <0.0007***, 2P <0.0001***

Data are expressed as mean ± SD, 1P: significance versus G1; 2P: significance versus G4. *: P <0.05, **: P <0.010, ***: P <0.0001. Significance was made using OneWay ANOVA test followed by Turkey test.

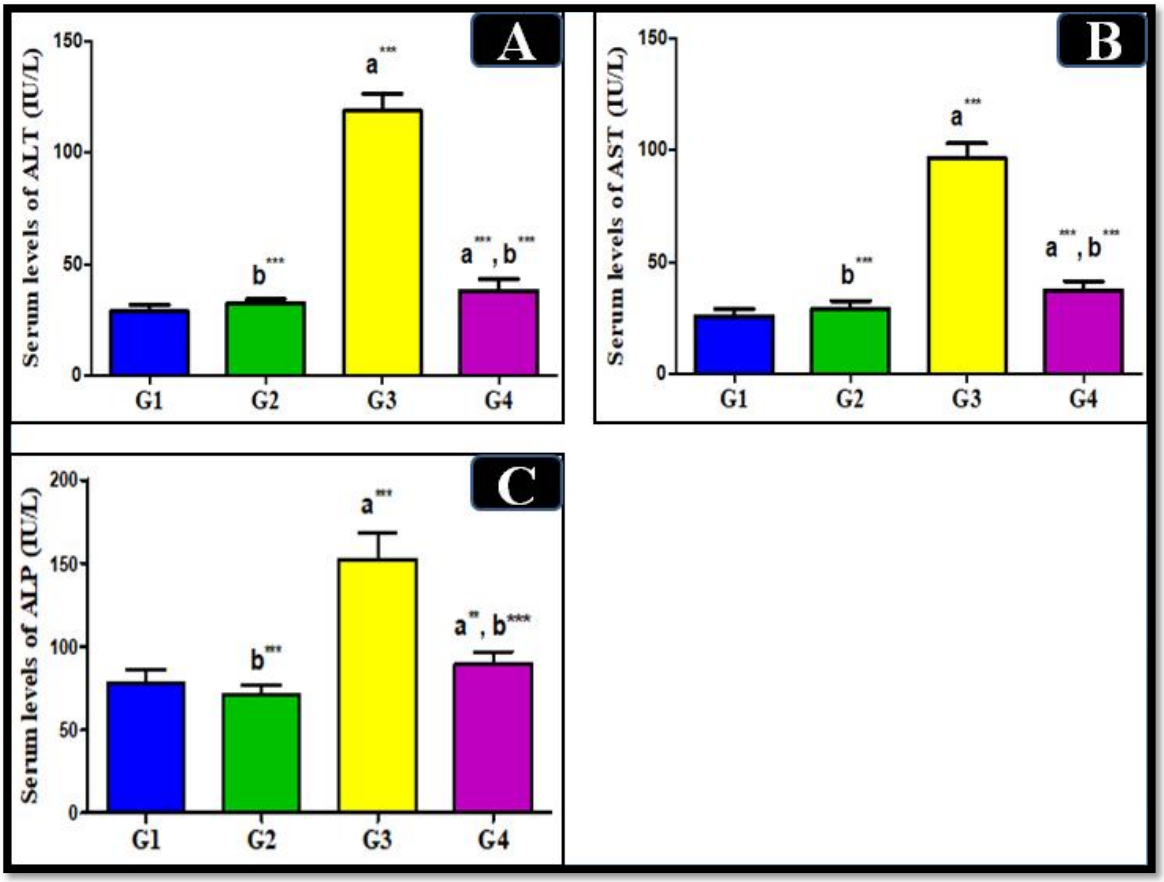


Fig. 2: Column charts showing the effect of TAA and DDO on (A) ALT serum levels (IU/L), (B) AST serum levels (IU/L), (C) ALP serum levels (IU/L) in different experimental groups. a: significance versus G1; b: significance versus G3.*: P <0.05, **: P <0.010, ***: P <0.0001. Significance was made using OneWay ANOVA test followed by Turkey test.

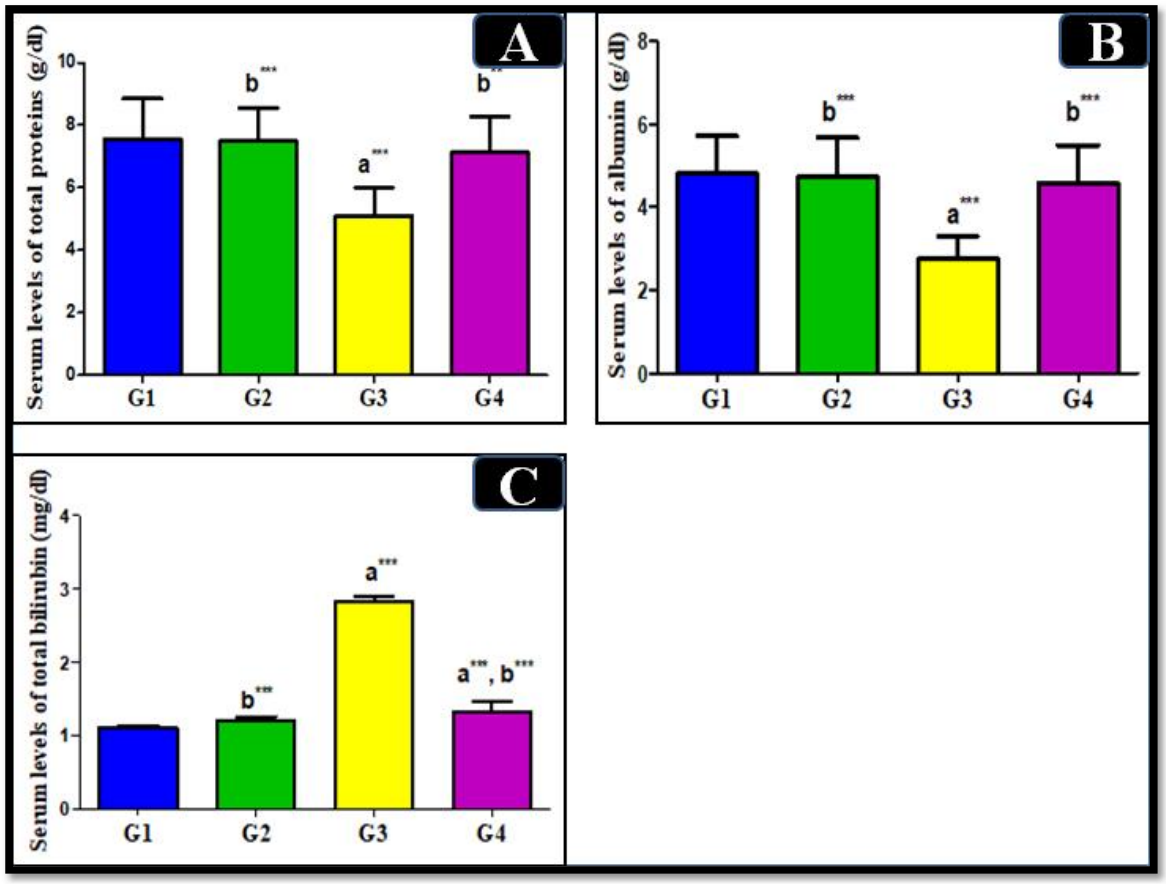


Fig. 3: Columnr charts showing the effect of TAA and DDO on (A) total proteins serum levels (g/dl), (B) albumin serum levels (g/dl), (C) total bilirubin serum levels (mg/dl) in different experimental groups. a: significance versus G1; b: significance versus G3.*: P <0.05, **: P <0.010, ***: P <0.0001. Significance was made using OneWay ANOVA test followed by Turkey test.

3.4. Liver oxidative and antioxidative markers

The data of MDA, the end product of lipid peroxidation and antioxidant enzymes; superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) is shown in Table 4 and Figure 4. In the TAA-intoxicated group, the level of MDA was a significantly increased (P<0001), while the antioxidant activities of SOD, CAT and GPx were significantly decreased compared with the control groups (P<0001). On contrast, when the rats were given DDO with TAA, there was significant decrease (P<0001) of MDA and an increase of SOD, CAT and GPx, respectively, when compared with the TAA-group alone group, indicating its antioxidant role.

Table 4: Effect of TAA and DDO treatments on rat oxidative stress mrkers in liver homogenates of different experimental groups

Groups	MDA	SOD (U/gm Tissue)	CAT (µmole/ gm Tissue)	GPx (µmol/ gm tissue)
Control group (G1)	18.04 ± 1.08	62.11 ± 3.31	196.16 ± 9.24	26.32 ± 3.1
DDO-treated group (G2)	17.88± 1.44	65.18 ± 3.52	188.14 ± 13.34	24.17 ± 2.7
Significance	2P <0.0001***	2P <0.0001***	2P <0.0001***	2P <0.0001***
TAA-treated group (G3)	56.34 ± 3.01	29.55 ± 2.05	99.46 ± 7.2	11.44 ± 1.9
Significance	1P <0.0001***	1P <0.0001***	1P <0.0001****	1P <0.0001***
TAA + DDO-treated group (G4)	21.78 ± 1.39	58.74 ± 3.36	182.19 ± 10.1	22.82 ± 2.8
Significance	1P <0.0001***, 2P <0.0001***	1P =0.0628, 2P <0.0001***	1P =0.012*, 2P <0.0001***	1P =0.0327*, 2P <0.0001***

Data are expressed as mean ± SD, 1P: significance versus G1; 2P: significance versus G4. *: P <0.05, **: P <0.010, ***: P <0.0001. Significance was made using OneWay ANOVA test followed by Turkey test.

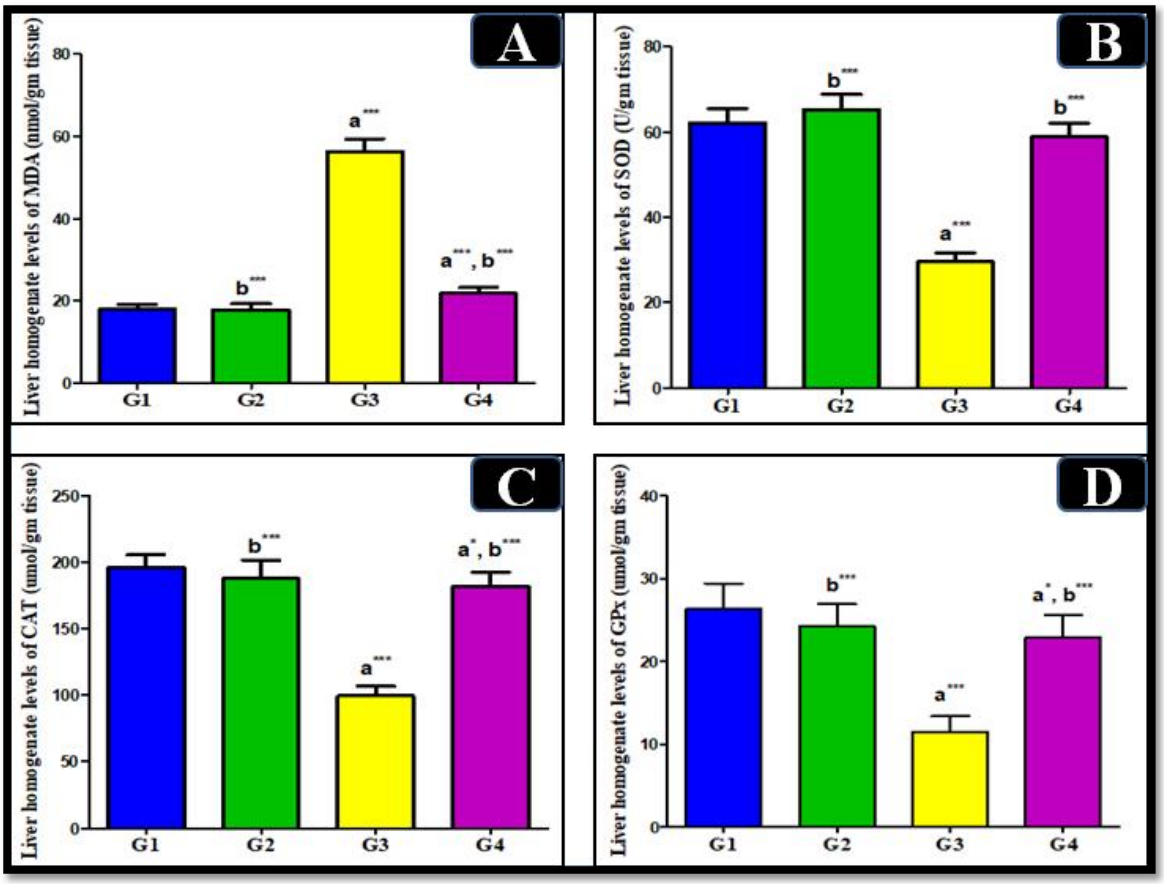
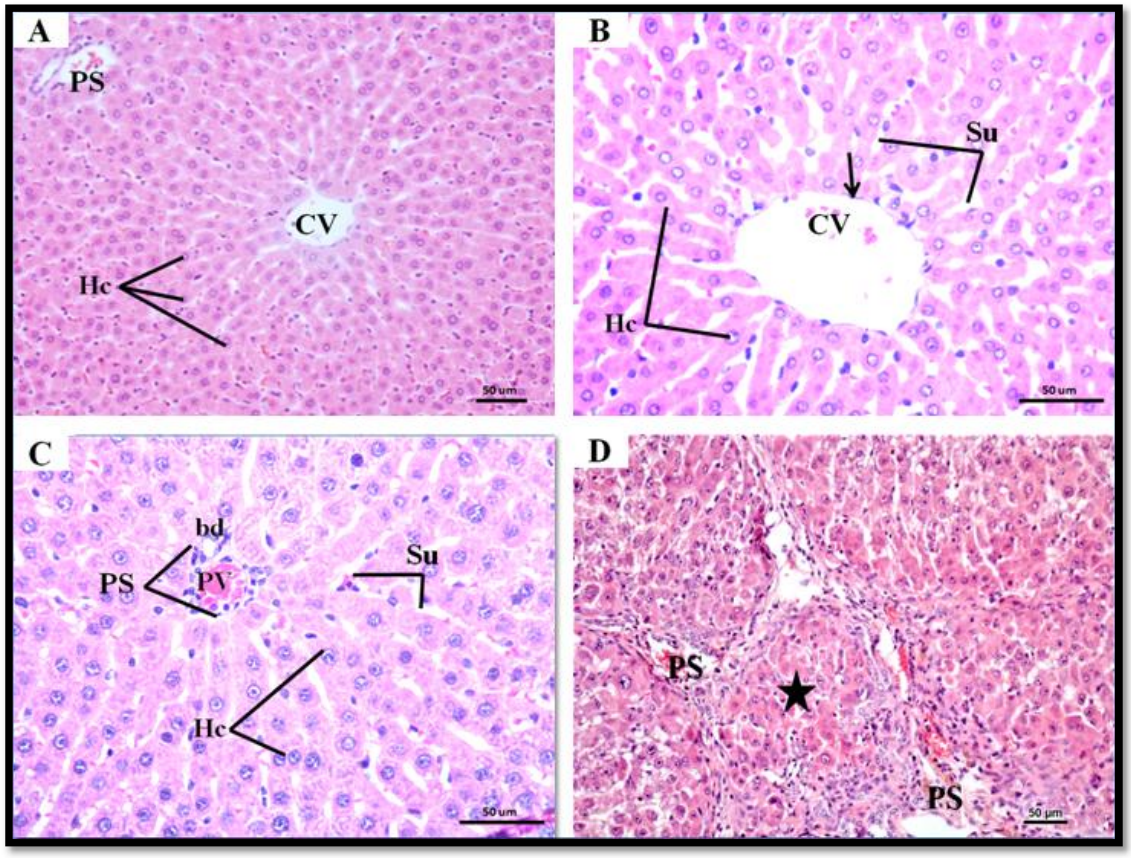


Fig. 4: Columnr charts showing the effect of TAA and DDO on (A) on MDA liver homogenate levels (nmol/gm tissue), (B) SOD liver homogenate levels (U/gm tissue), (C) CAT liver homogenate levels (umol/gm tissue), (D) GPx liver homogenate levels (umol/gm tissue) in different experimental groups. a: significance versus G1; b: significance versus G3.*: P <0.05, **: P <0.010, ***: P <0.0001. Significance was made using OneWay ANOVA test followed by Turkey test.

3.5. Histopathological results

The examination of H&E-stained liver sections from different groups showed that the control and DDO-treated groups (Figures 5 A, B, and C) displayed the normal hepatic architecture in the form of classical polyhedral lobules with portal spaces that were arranged at the peripheral angles of each lobule. The hepatic lobular parenchyma consisted of cords of hepatocytes radiating from the central vein towards the periphery. The spaces between the cell cords were occupied by narrow blood sinusoids that converged towards the central vein and were lined by flat endothelial cells. The hepatocytes had eosinophilic cytoplasm and contain contained one or two rounded nuclei. In the portal spaces, the small branches of the hepatic artery, portal vein and bile duct could be seen. In TAA-treated group, the examination revealed diverse forms of histopathological changes. In general, distortion of the liver parenchyma with loss of lobular structure, nodular formation and expansion of portal spaces were seen (Figures 5 D, E, and F).



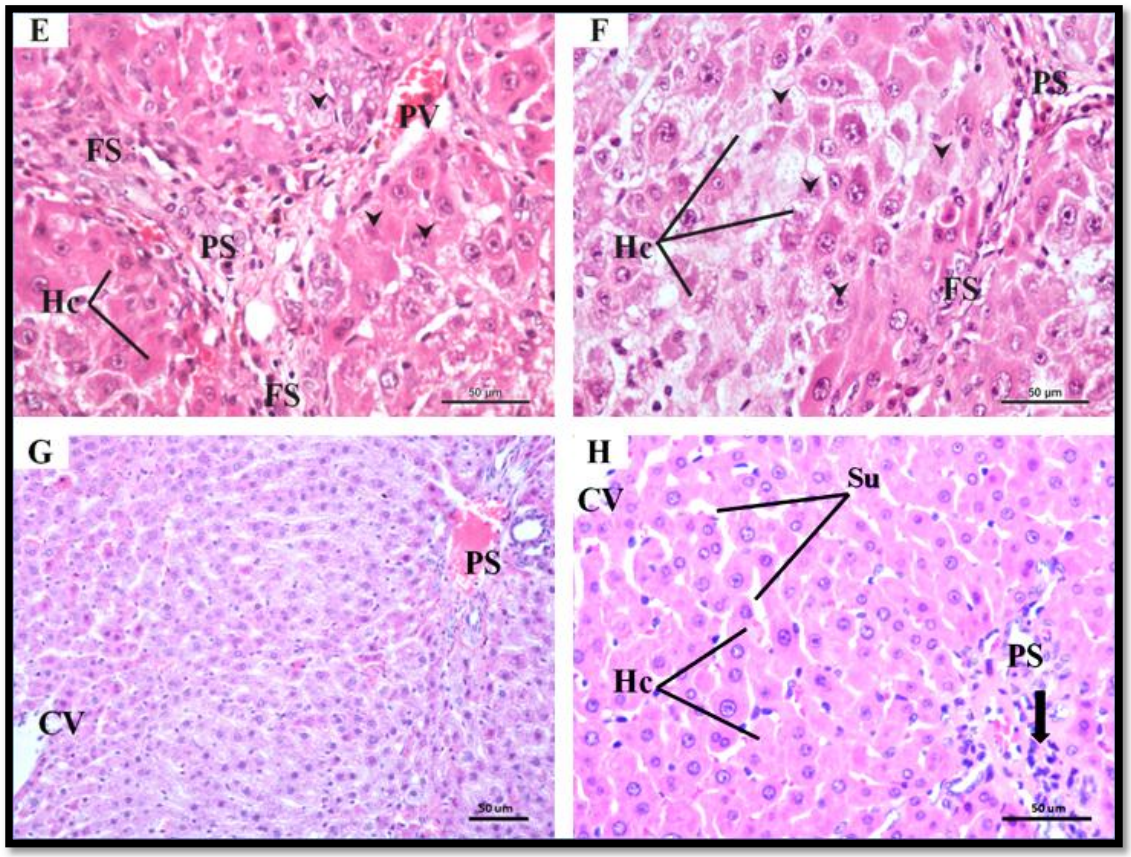


Fig. 5: Representative photomicrographs of H & E stained liver sections from different groups. (A, B, C) Control groups showing normal histological architecture of the liver including in the form of cords of hepatocytes (Hc) radiating from the central vein (CV) towards the portal spaces (PS). CV is lined by flat endothelial cells (↑). The hepatocytes had eosinophilic cytoplasm and contain one or two rounded nuclei. PS contains portal venule (PV) and bile ductule (bd). Notice normal hepatic sinusoids (Su). (D, E, F) TAA group showing loss normal histological architecture of the liver with of nodular appearance (star) between the portal spaces (PS), wich appeared dilated and gives rise to thickened fibrous septa (FS). Most hepatocytes (Hc) appeared vacuolated or swollen with their nuclei showed pyknotic changes (➧). (G, H) TAA co-treated with DDO group showing improvement of the lobular architecture with little dilation portal space (PS) and normally appearing hepatocytes (Hc). Notice a little dilation of some sinusoids (Su) and some inflammatory cells infiltration (thick arrow) in the portal space (PS). PV = portal vein. (A, D, G x 200 & B,C, E, H x 400)

There was disturbed continuity of the hepatic cords with dilatation of the blood sinusoids. Dilation and congestion of the portal venules with inflammatory cellular infiltration were detected in the portal spaces. The central veins appeared markedly congested and dilated with detached endothelial linings. Also, focal areas of parenchymal necrosis and inflammatory cellular infiltrates were observed in different hepatic lobules. The hepatocytes displayed a range of changes in the form of swelling and vacuolated cytoplasm and pyknotic changes of the nuclei, especially those in the centrilobular regions. The apoptotic hepatocytes showed nuclear irregularities, nuclear fragmentation and even nuclear loss. However, the examination of liver sections from the TAA co-treated with DDO (Figures 5 G, and H), revealed noticeable improvements in histopathological changes. There was a restoration of the lobular pattern with more organization of the hepatic cords. Also, there was diminished dilation of the portal spaces and portal veins. However, in some sections, a slight congestion was observed in the central veins and portal venules with slightly dilated blood sinusoids and inflammatory cell infiltration. The hepatocytes displayed mild degenerative cytoplasmic and nuclear changes.

The examination of the MT-stained liver sections from different groups showed that the control groups (Figure 6 A) displayed scanty fibrous tissue (stained blue) in the portal spaces and around the central veins. In the TAA-treated group (Figures 6 B and C), the examination revealed a marked increase of fibrous tissue in the portal spaces, which extended into the hepatic parenchyma in the form of bridging fibrosis and nodular formation. In addition, an increased amount of fibrous tissue was seen around the central veins and in different areas of the hepatic lobules. In contrast, the examination of liver sections from the TAA co-treated with DDO (Figure 6 D), revealed a noticeable decrease in fibrous tissue in the portal spaces, around the central veins, and around the hepatic lobules (mild septa radiating from portal tracts).

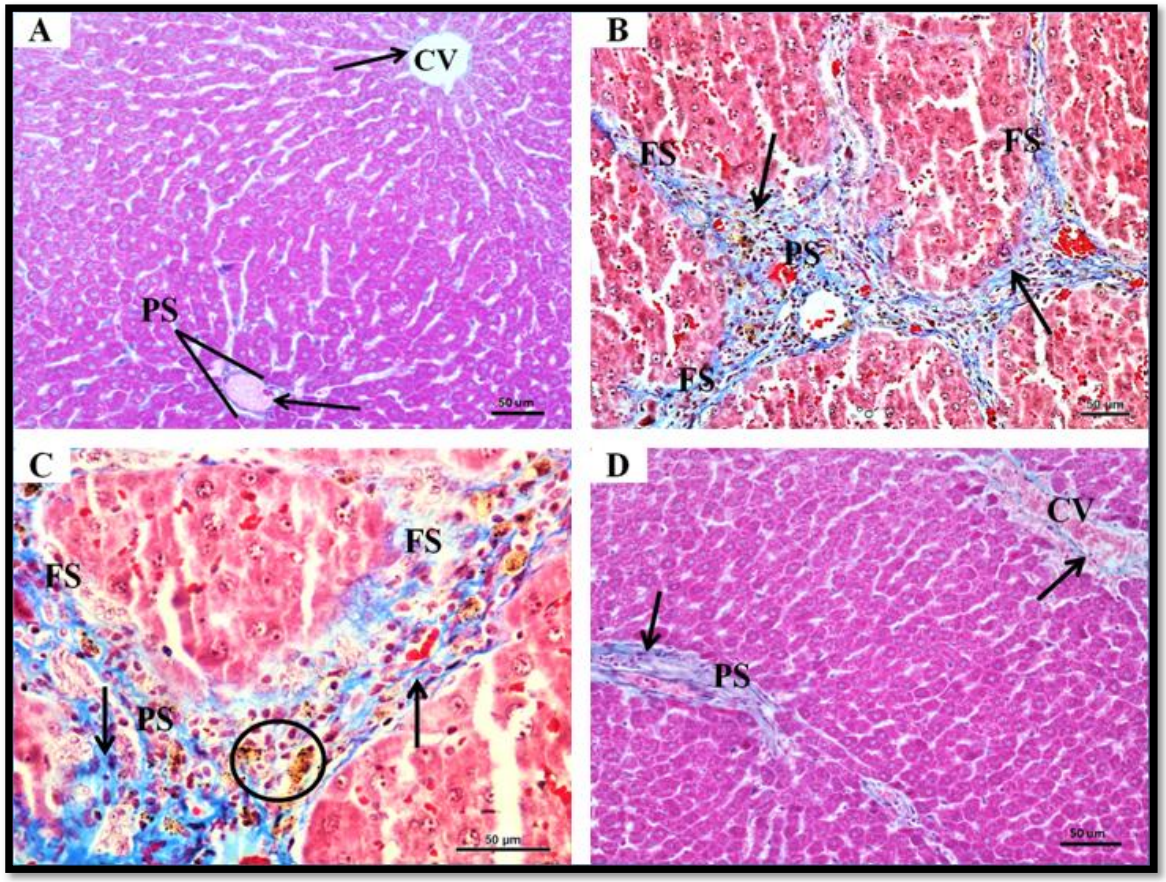
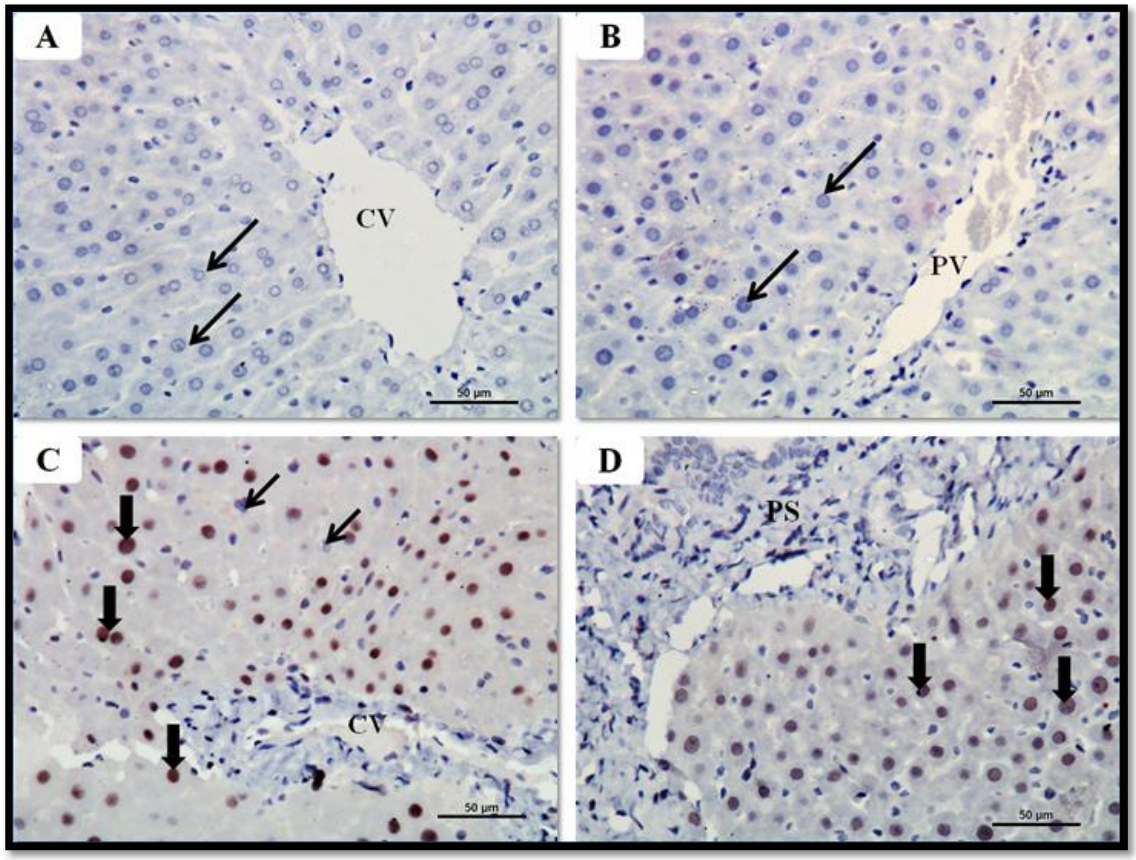


Fig. 6: Representative photomicrograph of MT-stained liver sections from different group. (A) Control groups showing scanty fibrous tissue (↑) (stained blue) in the portal spaces (PS) and around the central veins (CV). (B & C) TAA group showing marked increase of fibrous tissue deposition in the dilated portal space (PS) and in the fibrous septa (FS). Notice the presence of many macrophages (circle) in the porta space and fibrous septa. (D) TAA co-treated with DDO group showing decreased amount of fibrous tissue (↑) in the portal space (PS) and around the central vein (CV). (A, B, D x 200 & C x 400).

3.6. Caspase 3 immunohistochemistry

The immunohistochemical localization of caspase 3 was performed as a marker of apoptosis. The immunohistochemistry of caspase-3 in the liver sections from different groups showed that in the control groups (Figures 7 A and B), the hepatocytes displayed a very weak or minimal nuclear and cytoplasmic expression. In the TAA group (Figures 7 C and D), the hepatocytes exhibited strong and diffused nuclear and cytoplasmic expression of caspase-3 in many hepatocytes in the peri-central and peri-portal areas as compared to the control group, indicating the presence of many apoptotic changes. In the TAA co-treated with DDO group (Figures 7 E and F); caspase-3 was weakly expressed in the hepatocytes compared to the TAA group and had a nearly similar expression to the control group. However, weak expression was seen in some hepatocytes around the central vein. In the TAA co-treated with GSO group (Figures 16 G and H); mild to moderate caspase-3 expression in the hepatocytes was detected around the central veins and portal spaces.



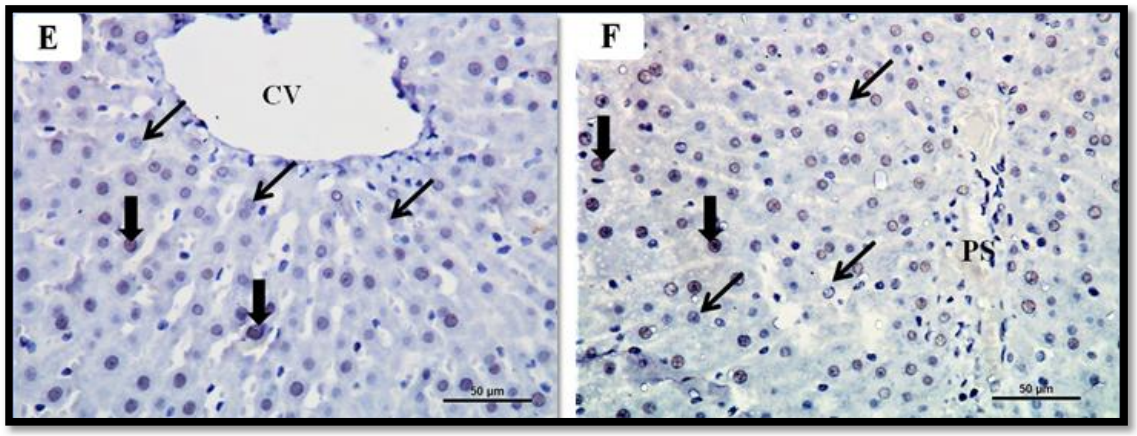


Fig. 7: Representative photomicrographs of caspase-3 immunohistochemistry in liver sections from different groups. (A & B) Control groups showing very weak or minimal nuclear and cytoplasmic expression in the hepatocytes (↑). (C & D) TAA-treated group showing strong expression (thick arrow) in many hepatocytes. (E & F) TAA + DDO-treated group showing weak expression in some hepatocytes (↑) around the central veins (CV) and portal spaces (PS). (×400).

3.7. PCNA immunohistochemistry

The liver regeneration was investigated by immunohistochemical identification of PCNA expression in proliferating liver cells. The nuclei of the hepatocytes labeled with anti -PCNA showed a positive reaction expressed as a granular or diffuse brown color indicating the presence of normal cell proliferation. The immunohistochemistry of PCNA in the liver sections from different groups showed that in the control groups (Figures 8 A and B), the hepatocytes displayed a very weak nuclear and cytoplasmic expression, representing the renewal occurrence. In the TAA group (Figures 8 C and D), the hepatocytes exhibited remarkable PCNA expression in many hepatocytes in the peri-central and peri-portal areas as compared to the control group, suggesting upregulation of these proteins with hepatic fibrosis. In the TAA co-treated with DDO group (Figures 8 E and F); PCNA was moderately expressed in the hepatocytes compared to the TAA group and had a nearly similar expression to the control group. However, weak expression was seen in some hepatocytes around the central vein.

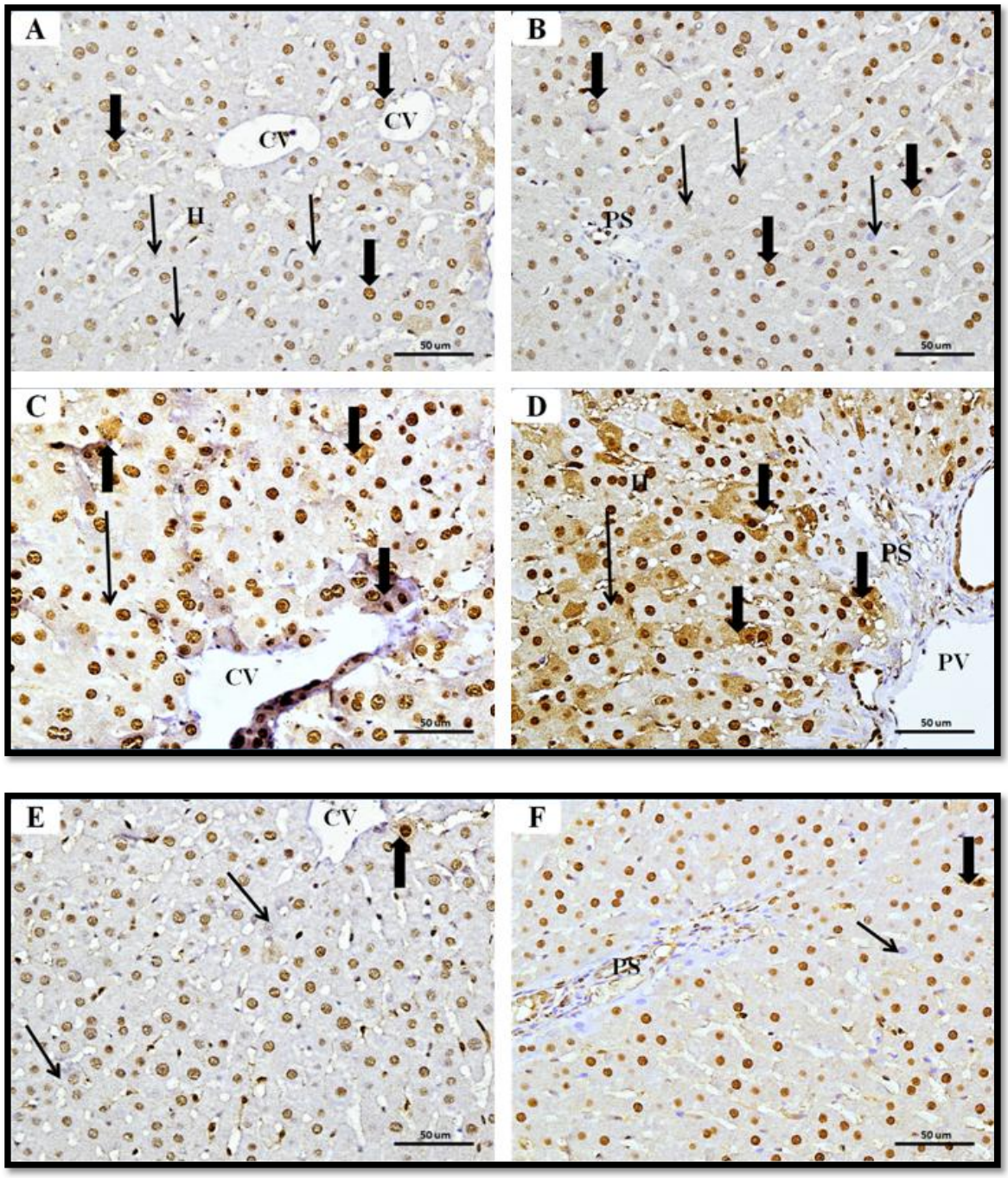


Fig. 8: Representative photomicrographs of PCNA immunohistochemistry in liver sections from different groups. (A & B) Control groups showing weak nuclear expression in the hepatocytes (↑) around the central vein (CV) and portal space (PS). Notice the presence of some hepatic nuclei with moderate PCNA expression (thick arrow). (C & D) TAA-treated group showing strong nuclear expression with cytoplasmic reaction in the hepatocytes (thick arrow) around the central vein (CV) and the portal space. Notice the presence of little hepatic nuclei with weak PCNA expression (↑). (E & F) TAA + DDO group showing moderate nuclear expression in the hepatocytes (↑) around the central vein (CV) and the portal space (PS). Notice the presence of one hepatocyte with strong PCNA expression (thick arrow). (×400)

4. Discussion

The main objective of this research was to test the possible protective role of DDO against experimentally induced liver fibrosis and hepatotoxicity in rats. The significance of this study is that in many people with severe liver disorders, liver fibrosis is the main problem, and when left without any treatment it finishes with cirrhosis. These conditions led to lower liver functions and the ultimate hepatic cell collapse that shadowed morbidity and mortality, thus raising the social and economic strain of these patients. In addition, in many places across the world, this is a major health problem because irreversible liver damage contributes to cirrhosis and eventual liver failure, a disease unavoidable and without sufficient attention (World Health Organization, 2019).

In several trials, liver fibrosis is a complex, combined molecular and cell mechanism that has been well observed in humans as well as laboratory animals and, as such, has been called a reversible process (Bae *et al*., 2018; Podgórska *et al*., 2020; Ezhilarasan and Najimi, 2021; Zhao *et al*., 2021). Other studies have nevertheless shown that the complete regeneration is not accomplished and the liver cells stay on the verge of reactivating quickly into myofibroblasts, as deteriorating stimuli occur and a more serious level of fibrosis is promoted (Berumen *et al*., 2020; Marcelin *et al*., 2020; Yavuz *et al*., 2020). Furthermore, severe liver disease decreases the hepatocyte regenerative potential, which becomes inadequate, as well as apoptosis or necrosis. This results in excessive fibrous tissue deposition, which deforms the hepatic tissue's architecture and can trigger liver malfunction (Trautwein *et al*., 2015; Tsuchida and Friedman, 2017; Schuppan *et al*., 2018; Dhar *et al*., 2020).

Cellular and molecular mechanisms that contribute to hepatic fibrosis growth and progression have a range of treatments, including the development of hepatic fiber treatment and the development of pharmacological methods that can modify the mechanism of fibrosis (Higashi, 2017; You *et al*., 2018; Lafoz *et al*., 2020; Prestigiacomo *et al*., 2020; Zhou *et al*., 2021).

In this research, the model of TAA-induced fibrosis in a rat has been used to examine the fundamental mechanisms of hepatic fibrogenesis, as well as the therapeutic consequences of possible antifibrotic products due to their reproducibility, ease of usage, and prolonged injury and recovery patterns (Alomar and Al-Attar, 2019; Mettwally *et al*., 2021; Yan *et al*., 2021). TAA-mediated liver fibrosis is intimately comparable in contrast with other animal models of alcoholic liver fibrosis, with related histological and metabolic disorders typically detected in human livers (Algandaby, 2018; Carlessi *et al*., 2019). Ideally, each model of liver fibrosis should embody the main clinical and molecular characteristics of the condition, including parenchymatic centralised fibrosis in chronic hepatitis. In addition, *in vivo* liver fibrosis models should be simple to implement and reproducible (Sung *et al*., 2013).

In this study, the rats received intraperitoneal injections of TAA in a dose of 200 mg /kg body weight (b.wt.) by intraperitoneal injection twice weekly to induce liver fibrosis, which accorded with previous studies (Hsieh *et al*., 2008; Aydin *et al*., 2010). Moreover, separate studies have shown that in various models of animal experiments, TAA resulted in liver damage, fibrosis, and cirrhosis (Al-Attar *et al*., 2016; Al-Attar and Al-Rethea, 2017; Luo *et al*., 2018; Abood *et al*. 2021).

The present results showed the least increase in body weight in TAA-treated rats accompanied by a notable increase in liver/body ratio as compared to the control. Other researchers who considered this decreased body weight gain to be the result of the direct toxicity of TAA or indirect toxicity related to liver damage (Wong *et al*., 2012; Kadir *et al*., 2013; Kabiri *et al*., 2014; Zargar, 2014). In explanation, it was reported that the decrease in body weight gain recorded in TAA treated rats may be related to malnutrition resulting from reduced absorption of nutrients from the intestine. Additionally, this observed reduction could be due to the direct effect of TAA on the food intake behavior of the rats and the concomitant loss of animal appetite with subsequent reduction of food ingestion (Al-Attar and Shawush, 2015; Al-Attar *et al*., 2016).

In this experiment, the TAA-treated rats showed an increase in liver body weight relative to control rats, indicating an expansion of the liver related to the toxicological effects of TAA. In comparison to liver weight alone, the estimation of hepatic hepatitis was seen to be a more reliable method to detect improvement in liver size as the hepatic weight relies mostly on the size of rats (Lin, 2017). According to several previous studies, the increased liver weight was probably related to the accumulation of ECM-proteins such as collagen which was caused by TAA-induced liver toxicity (El-Baz *et al*., 2020; Jantararussamee, 2020; Na *et al*., 2021). Likewise, some researchers employed

plentiful extracts from medicinal plants and demonstrated a decrease in the ratio of weight/body to hepatotoxicity in the liver (Salama *et al*., 2018; Urrutia-Hernández *et al*., 2019; El-Hawary et al., 2020).

In this observation, the application of TAA to rat, is shows that serum ALT, AST, ALP and total bilirubin were significantly higher than those of control rats, as their leakage in the blood from damaged hepatocytes. Findings also correspond to earlier studies, which demonstrate that the impact of TAA on liver cell membranes is oxidation effects combined with a lack of cytoplasmic enzyme blood stream volumes such as ALT and AST, which are related to liver problems (Salama *et al*., 2017; El-Baz *et al*., 2019; Sayan *et al*., 2020). This was also confirmed by other studies that reported a significant increase in these parameters which might be due to hepatocyte necrosis and inflammation induced by TAA (Al-Attar and Shawush, 2015; Al-Attar *et al*., 2016; 2017; Sukalingam *et al*., 2018; Hosseini *et al*., 2020; Tsai *et al*., 2021). Moreover, it was reported that the increase in serum total bilirubin may be due to blockage of bile ductules as the result of inflammation and fibrosis in the portal triads or to the regurgitation of bilirubin from the necrotic hepatocytes to the sinusoids (de David *et al*., 2011). In addition, the elevation of ALP, a cell membrane enzyme is a primary marker of hepatobiliary effects and cholestasis (Zhao *et al*., 2020).

A substantial decrease in overall serum protein and albumin levels following TAA injections has been seen in current research findings. These findings were consistent with previous studies, which indicated that the decrease could be attributed to inflammatory reactions or to perturbed protein biosynthesis in the fibrotic liver (Alshawsh *et al*., 2011; Salama *et al*., 2013; Hessin *et al.*, 2015). In addition, the TAA poisoning was documented to contribute to serious harm to the polyribosoms of the endoplasmic hepatocyte reticulum, contributing to protein synthesis depression. (Lin *et al*., 2017; Zhan *et al*., 2019; Su *et al*., 2020).

However, the present data showed that DDO and TAA administration resulted in a substantial improvement in the markers of hepatic damage and improved metabolic function as described by plasma proteins and bilirubin. It was thought that DDO would protect the membrane structure of hepatocytes and avoid cell damage induced by TAA and its metabolites, indicating the repair of damage caused by hepatic tissues (Bayoumy *et al*., 2015; Mohamed, 2018; Meda *et al.,* 2020).

TAA injections into rats led to considerable oxidative stress in the rat liver, manifested by a considerable rise in the levels of MDA, a lipid peroxidation end product, and a substantial decrease in the levels of SOD, CAT, or GPx antioxidants. Such findings agreed with Chen *et al*. (2015) who confirmed that increased MDA levels in the liver are caused by free radical-mediated lipid peroxidationafter a TAA injection, which resulted in tissue injury, mitochondrial dysfunction, and failure to scavenge excess free radicals by the antioxidant protection mechanism. Moreover, Kaur *et al*. (2017), who reported that low concentrations of SOD and CAT have been implicated in TAA-induced liver fibrosis and cell injury.

Many investigators have also extensively documented that oxidative stress is a key process that leads to liver damage and the initiation of liver fibroids, resulting in an altered balance between cell oxidant and antioxidant factors, resulting in overproduction of reactive oxygen species from various sources, including the epithelium, activated inflammatory cells, and microvascular endothelium. ROS further stimulates the synthesis of pro-inflammatory and pro-fibrogenic agents through the use of HSCs, Kupffer, and other inflammatory cells (Liu *et al*., 2010; Cichoż-Lach and Michalak, 2014; Wang *et al*., 2014; Sato *et al*., 2016; Yuan *et al*., 2017; Zoubek *et al*., 2017).

The procedure with DDO was found in this analysis to improve SOD and CAT and the GSH content substantially. This oil can also play an antioxidant role in hepatic fibrosis by aiding in the recovery of the liver's natural antioxidant protection mechanism. The protection impact of DDO is believed to be linked to its free radical scavenger and antioxidant function, which decreases TAA-mediated ROS generation and oxidative stress. Different antioxidants have been shown to avoid or treat hepatic injury (Nauman *et al*., 2020; Gavzan *et al*., 2021).

The present findings suggested that administration of TAA in rats contributed, in the form of structure disorder, to a significant level of histopathologic liver injury, extraordinary fibrotic fibrosis, fibrotic septa appearance and nodular lobules. According to present results, TAA caused serious hepatic harm, as shown by histopathology combining significantly higher levels of liver markers: ALT, AST, ALP, GGT, and total bilirubin, which are similarly illustrated by Lee *et al*. (2019); Zeyada *et al*. (2020). Moreover, it was reported by Alomar (2020); Renovaldi and Adam, (2020) that

the addition of TAA resulted in an increased amount of fibrous tissue surrounding lobules, which formed fibrous tissue distorting septa, and triggered the activation of HSC and genetic overexpression of lipid peroxidation fibrogenic cytokines. Similarly, it was documented that TAA administration resulted in great damage to the rat liver represented by the nodular appearance of the liver lobule, bridging necrosis, and vascular degeneration of hepatocytes (Chen *et al*., 2012; Feng *et al*., 2018).

In this research, rats' livers treated with TAA demonstrated hepatocyte degeneration with cytoplasmic vacuolar and periportal infiltration of cells with severe centrilobular necrosis and blood sinusoidal and central vein obstruction comparable to the results of Emam *et al*. (2018). In addition, Salama *et al*. (2013) have described the hepatic damage after TAA administration that ranged from parenchymal cell necrosis and liver cell proliferation, to the production of pseudolobules and nodular cirrhosis. In similar study Cruz *et al*. (2005) noted that TAA induces liver fibrosis, oxidative stress with substantial tissue destruction, and improved expression of alpha smooth muscle actin in rat liver to explain the histopathological damage.

Furthermore, l-Attar (2012) reported that the levels of liver SOD and GSH were significantly decreased in mice treated with TAA. Similarly, Luo *et al*. (2015) have illustrated that the level of liver MDA was significantly elevated in TAA-treated rats while the contents of SOD and GSH decreased compared with the control rats. Moreover, Mansour *et al*. (2015) display a decline in GSH and SOD activity and the nitrogen oxide levels in rats. The authors demonstrated that TAA-mediated hepatic oxidative damage as seen in increased lipid peroxidation. On the other hand, this analysis shows that DDO therapy reduces histopathological changes in rats due to TAA. This showed the efficacy of DDO in TAA toxicity avoidance. A potential hepatoprotective factor function of the studied DDO can be caused by its antioxidant effect, which impairs the reactive activation of TAA (Amin *et al*., 2012; Salama *et al*., 2013b; Kaur *et al*., 2019).

In this study, a caspase 3 immunohistochemical test, which is a main factor in apoptosis, was conducted. An apoptosis marker was then considered with extreme caspase 3 activation of apoptotic cells (Ozaki Michitaka *et al*., 2012; Mohamed and Magdy, 2017; Yang Min *et al*., 2017; Guicciardi Maria Calabrò *et al*., 2020). The finding of the current study indicated that caspase 3, TAA-treated rats had intense reactions, which showed high apoptosis in relation to the control group. In accordance, it was reported by Bantel *et al*. (2004; Abdel-Salam *et al*. (2005) that elevated caspase-3 expressionas a marker of apoptosiswas observed in the liver of TAA-treated rats, where aspase-3 is a sensitive marker reflecting liver damage and is associated with liver. Similarly, TAA has been confirmed to cause major necrosis of the liver and thus trigger oxidative stress in caspase-3, increasing apoptosis. In addition, elevated apoptotic hepatocytes in hepatic fibrosis and viral hepatitis have been recorded (Omneia *et al*., 2014; El Awdan *et al*., 2018; El-Kashef and Serrya, 2019; Sayan, *et al*., 2020).

There are many modulators that could regulate the apoptosis, including ions (e.g., calcium), genes (e.g., c-myc, Bcl-2/Bax and Fas), proteins (e.g. p53, caspases, IAPs) and even organelles (e.g., mitochondria, endoplasmic reticulum) (Portt *et al*., 2011; Ulukaya *et al*., 2011). In the activation of apoptosis, oxidative stress and inflammatory pathway induction were already indicated (Kuwahata *et al*., 2012). The results of this study showed that co-treatments of DDO and TAA substantially reduced the expression of caspase- 3 in the liver cells, indicating the defensive and anti-apoptotic activity of DDO. The inactivation of caspases has been shown to shield hepatocytes from apoptosis, reduce cytokines involved in inflammatory signaling and improve fibrogenesis (Dixon *et al*., 2012).

In addition, an immunohistochemical analysis has been carried out of PCNA expression as a measure of proliferation and regeneration of liver cells (Albrahim and Binobead, 2018; Zhou *et al*., 2020). In comparison to the control group, PCNA-positive hepatocytes were found to be quite large in TAA. In the periportal cities, the positive hepatocytes were deeper than in the core veins. The only organ that shows an impressive capacity for regeneration after damage or surgical resection in the mammalian body is well known to be the liver (Inomata *et al*., 2000). When liver regeneration is compromised, it may lead to liver failure pathogenesis or the production of fibrosis, which is seen as a general answer to different liver injuries (Julien *et al*., 2005; Wynn, 2008). It has been documented recently that in the TAA community, up-regulation of hepatocytes of the appearance of PCNA, which represents large multiplication, and potential tissue injury repair effort, was observed (Abood *et al*., 2020). On the other hand, rats fed slightly lower cell proliferation with DDO as PCNA expression decreased. In experimental TAA-induced liver cirrhosis animals fed with medicinal plant extracts,

prior down-regulation of PCNAs was observed (Salama *et al*., 2013a; Bardi *et al*., 2014; Kadir *et al*., 2014).

To date, liver diseases, including fibrosis and cirrhosis, were not adequately treated; no hepatic disease progression was effectively inhibited. Conventional medicines for the treatment of hepatitis are often insufficient and can have severe negative consequences, which remain a therapeutic challenge (Byun *et al*., 2021; Ng *et al*., 2021). New therapeutic liver fibrosis strategies are therefore developed to identify antioxidant compounds that improve oxidative status, avoid the free generation of radicals to inhibit ROS-mediated fibrogenesis, and reduce the development of liver fibrosis (Gbian and Omri, 2021; Wang *et al*., 2021). In addition, the ideal antifibrotic medication was reported as being easy to administer, well tolerated, with high liver specificity, and encouraging excessive tissue resorption without decreasing the beneficial effects of normal hepatic fibrous tissue (Weiskirchen *et al*., 2018; Mauri *et al*., 2021).

In this context, numerous studies have demonstrated the protection of the liver through the antioxidant action of several herbal products, which plays an important role in protecting cells and tissues against stress loss due to the oxidative effects (Bjelakovic *et al*., 2011; Heidari *et al*., 2018; Ansari *et al*., 2019; Jia *et al*., 2019; Radi *et al*., 2021; Shafaei *et al*., 2021).

In this report, DDO and TAA approved an effective antioxidant and antifibrotic agent to restore almost the usual liver architecture. The appearance of flavonoids and other bioactive components seen in the extract can be due to this reality. The flavonoids serve as antioxidants and may lead, among other pharmacological activities, to the hepatoprotective action of plants (Kaur *et al*., 2018; Urrutia-Hernández *et al*., 2019). Previous research showed that *Balanites aegyptiaca* extract has high concentrations of flavonoids, alkaloids, and saponins that display a variety of pharmacological and biochemical behavior (Ifedi *et al*., 2017; Safiya *et al*., 2018). Moreover, Attia and Al-Radadi (2016) showed that *Balanites aegyptiaca* extract has been confirmed to avoid liver fibrosis via the suppression of genotoxicity and inflammatory pathways and the increase in collagen degradation.

This study concluded that DDO has hepatoprotective and anti-fibrotic effects on liver fibrous activity by minimising the TAA-induced biochemical, oxidative, and histopathologic effects in rats, possibly as a result of their antioxidant characteristics and enhanced propagation and reducing hepatocyte apoptosis in rats due to their phytochemistry. The findings finally verified the possible protective therapy of the desert date and oil that is promising for liver fiber therapy caused by several chronic diseases of the liver, which may be highly clinical in antifibrotic liver treatment. However, further analysis of hepatoprotective active compounds is needed to isolate, characterize, and function as a mechanism.

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