Ameliorative Effects of Pomegranate Molasses and Fig on Carbon Tetrachloride Hepatotoxicity in Rats

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الجزء الأول

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Abstract :

The possible preventive effects of pomegranate and fig against hepatocellular toxicity induced by carbon tetrachloride (CCl_4) in male rats were studied. The pomegranate molasses and fig indicated the existence of flavonoid and phenolic components in their entirety. Each of the eight groups of rats contained six rats; group (1) served as the negative control, or normal group, and the rats in the other groups received subcutaneous injections of carbon tetrachloride (CCl_4) twice weekly for six weeks to induce hepatocellular toxicity. Group (2) was left as a positive control, while the rest groups were administered with (2.5%, 5% and 10%) pomegranate molasses or dried fig , for 6 weeks, respectively. Blood samples were collected at the conclusion of the experiment for biochemical examination. The results showed that, in comparison to the normal control group, the rats with hepatocellular toxicity had significantly higher serum levels of AST, ALT, total protein, creatinine, and blood urea nitrogen. The administration of (2.5%, 5% and 10%) pomegranate and dried fig preserved liver caused a significant reduction in serum levels of AST, ALT, total protein, creatinine and blood urea nitrogen while an increase in hemoglobin and RBC was greater in pomegranate especially (10%) group. This study concluded that pomegranate and dried fig could be implemented in the protective effect of hepatotoxicity in rats and resume the anemic effect caused by CCl_4. Therefore this study recommends Increased dietary intake of pomegranate and dried fig may be beneficial for patients with liver and kidney diseases as a preventative measure.

Keywords: PomegranateMolasses;Fig;CarbonTetrachloride; Hepatotoxicity ; Liver enzymes; Kidney Function; Rats.

Introduction

Oxidative stress is brought about by a decrease in antioxidant defense mechanisms or the incapacity of different antioxidant mechanisms to scavenge excessive amounts of reactive oxygen species (ROS). Thus, degenerative illnesses such as hepatopathies (Hensley *et al.*, 2000) and nephropathies (Ateşşahin *et al.*, 2003) might happen. The first tissues to suffer from oxidative stress brought on by drugs, alcohol, pathogenic agents, hazardous industrial chemicals, dietary additives, and air and water pollution are the liver and kidneys. Moreover, the development and advancement of cancer and liver disease are significantly influenced by free radicals and ROS(Jemal *et al.*, 2007).

An industrial solvent called carbon tetrachloride (CCl_4) is widely utilized as a xenobiotic to cause chemical liver harm. In mouse models, CCl_4-induced oxidative stress is frequently utilized to assess whether synthetic or natural compounds provide protection against drug-associated hepatotoxicity and nephrotoxicity (Haghi *et al.*, 2014).

The Pomegranate (Punica granatum) name has been verified as appropriate by the websites "MPNS" and "World Flora Online." Alvarez-Cervantes et al. (2021) state that the pomegranate's exceptional qualities can be traced back to its historical endemic countries, which date back to the early Bronze Age (3500–2000 BC). This plant is indigenous to the Himalayas, and according to botanical data, the first areas where humans cultivated pomegranates were in central Asia, which includes countries that stretch from spanning the Middle East, the European Mediterranean region, northern Africa, and Iran and northern India (Abdel Moneim, 2012; Vučić *et al.*, 2019). But nowadays, It is also grown in China, Greece, Italy, Morocco, Spain, Russia, North and South America, and Uzbekistan (Sohrab *et al.*, 2015, 2017; Vučić *et al.*, 2019). The most widespread religions in human history, such as Islam, Judaism, Zoroastrianism, and Christianity, along with old folktales that depict pomegranates as symbols of life, femininity, and immortality (Akhtar *et al.*, 2017). The pomegranate is native to the Middle East (Kandylis & Kokkinomagoulos, 2020).

One of the most well-known traditional edible plants is the pomegranate(Xie *et al.*, 2008). It is described as the "Food of Gods," signifying plenty, fruition, and success, in the Bible, the Torah, the Islamic holy book (Quran), and the Babylonian Talmud (Seeram *et al.*,

2006). Pomegranate (Punica granatum L.) have a wide range of beneficial phytochemicals, including tannins, alkalioids, and colors (Zarei *et al.*, 2017), It is a plant used in popular folkloric medicine for the treatment of various diseases (Ajaikumar *et al.*, 2005). It is an important source of organic acids, anthocyanins, flavonols, proanthocyanidins, hydrolysable tannins punicalagin and punicalin (Vučić *et al.*, 2019 & Hou *et al.*, 2019 & Afaq *et al.*, 2005), ellagic and gallic acids (Raffaele *et al.*, 2021) and contains vitamin C (Turk *et al.*, 2008). Recent studies have demonstrated its anticancer activity (Kumar and Das, 2022).

Numerous studies on the in vivo characteristics of pomegranates, namely their anti-atherosclerotic capacity, have been published in addition to their antioxidant capacity (Rosenblat *et al.*, 2015). It have antioxidant properties because of phenolic components such phenolic acid and flavonoids (Moradnia, et al., 2024).

Howell & D'Souza, (2013)reported that pomegranate juice possesses antiviral properties that prevent viruses from attaching to host cell receptors. Tomás-Barberán *et al.*,(2017) demonstrated that gut microbiota-derived urolithins and punicalagins, in particular, have anti-inflammatory properties that are mediated by the peroxisome proliferator-activated receptor transcription factors (PPAR), which inhibit the activation of nuclear factor κB (Feng *et al.*, 2020 & Ciavarella *et al.*, 2020). Furthermore, new research indicates that some components of pomegranate peel extract may be able to protect against SARS-CoV-2 (Suručić *et al.*, 2021), anti-carcinogenic (Khan, 2012), anti-inflammatory properties (Stefanou, *et al.*, 2020), as well as therapeutic efficacy (Peñalver-Mellado *et al.*, 2023).

In the Middle East, pomegranate molasses (PM), a concentrated form of pomegranate juice, is commonly consumed and may contain more potent antioxidants than the juice itself. It is a concentrated product made simply by boiling the juice, and it is often used in salads and many other foods to improve the taste and scent qualities in Egypt (Abd Elmonem, 2014).

Fig is referenced in Surah Al-Tin: 1-2 of the Holy Quran where God Almighty takes the form of a holy oath and says, "By the fig and the Olive, and by Mount Sinai." Given the significance of this scripture, God Almighty vowed to fig. The Ficus carica genus has over 800 variants that are grown in warm climates. Figs are a seasonal fruit that are good either fresh or dried, or in juice or jam. They can be collected twice a

year. The fig tree, is one of the oldest crops ever domesticated. It comes from the Middle East and belongs to the family Moraceae (Mulberry). The fruit is currently being grown in various parts of the world because of its health, nutritional value, and economic significance (Abdel-Rahman *et al.,* 2021; Wojdyło *et al.,* 2016). It can be eaten raw, dried, or processed (Dueñas *et al.,* 2008). The main producers of figs in the west and east Mediterranean region include Turkey, Egypt, Tunisia, Iran, Morocco, and many other countries. Fig production has increased in Brazil, the US, China, Portugal, and other countries (Veberic and Mikulic-Petkovsek, 2015).

The most prevalent colours of fig fruit (Ficus carica) are purple and green, though the exact colour varies based on the subspecies to which it belongs and the planting region. Furthermore, because fig trees are employed as objects of religious and cultural worship throughout the regions of the world where they originated, they have enormous cultural and religious value (Badgujar *et al.,* 2014). Because of their appealing sensory and organoleptic features as well as their capacity to promote health, they have good economic potential in addition to their use in a variety of cultural contexts. Scientific study has provided empirical evidence regarding the significance of fig fruits and leaves, particularly in relation to human diets and health. Various tree parts have been found to have substances and qualities that promote health and are effective against a number of known lifestyle disorders (Abdel-Rahman *et al.,* 2021; Amessis-Ouchemoukh *et al.,* 2017).

This is further supported by the fact that the traditional Ayurvedic and Siddha medical systems, which are popular in India and other Middle Eastern nations, have already shown the medicinal value of this tree (Badgujar *et al.,* 2014).

Numerous fig tree sections have been investigated and found to have health-promoting properties in the past. For instance, it has been shown that bioactive chemicals found in fig seeds possess anti-oxidant qualities and can help the body's oxygen imbalances (Nakilcioğlu-Taş and Ötleş, 2021). This could be explained by phytosterol and its constituents, including stigmasterol and β-sitosterol, which have the ability to decrease cholesterol (Barolo *et al.,* 2014; Jeong and Lachance, 2001).

Apart from the seed, Boyacıoğlu et al. (2021) and Wojdyło et al. (2016) have reported the potential therapeutic benefits of fig fruit and leaf against chronic and lifestyle disorders, including diabetes and cancer. An earlier study on the anticancer properties of fig leaf extract,

showed 67–80% inhibition of the Hep2 and HepG2 cell lines (Abdel-Rahman *et al.,* 2021). Boyacıoğlu et al. (2021) have reported on the promising of latex-derived antiproliferative agents from Ficus carica on several types of HT-29 cancer. Mild constipation can be treated with the syrup (George *et al.,* 2023).

Several research (Al-Snafi, 2017; Chauhan and Tanwar, 2015; Khan et al., 2011; Pal, 2020; Sadia et al., 2014; Verma et al., 2015; Vora et al., 2017) reported that carotenoids, organic acids, vitamin E, and phenolics are present in the fruit of fig and it is a great source of phytochemicals that improve health. Figs are a nutrient-dense fruit Different fruit kinds, their state (fresh or dry), their location, the type of soil they are in, and the overall climate of the fruit's surroundings all affect the amounts and concentrations of these micro and macronutrients. Figs are high in minerals like iron, calcium, phosphorus, potassium, and sodium, which are required for the physiological functioning of the human body (Al-Snafi, 2017; Chauhan and Tanwar, 2015; Mahmoudi *et al.,* 2018; Pal, 2020).

The dried fruit has been shown to contain a high concentration of carbs, ranging from 65.2% to 73.5%, but fresh fig fruits contain only 8-20% (Pal, 2020).

Fruit, latex, leaves, and roots all include volatiles, aliphatic alcohols, fatty acids, hydrocarbons, sterols, anthocyanins, and other secondary metabolites. Furthermore, figs are a vital source of vitamins (riboflavin and thiamin) and minerals (including potassium, iron, and calcium). Fresh fruit from Ficus carica, extracts, and extracted bioactive components showed a wide range of health-promoting characteristics. Ficus carica peel extracts contained a variety of nutrients and bioactive substances, such as fatty acids, organic acids, tocopherols, phenolic components, and free sugars (George *et al.,* 2023).

Ficus carica(FC) is recognised for its biological and health properties, which include antifungal, antihelminthic, acetyl cholinesterase inhibition, and anticarcinogenic activity. These properties are found in its leaves, roots, fruit, and latex. Traditional medicine uses fig to treat a variety of respiratory, gastrointestinal, endocrine, and reproductive conditions. It is applied to infections of the urinary and gastrointestinal tracts. In addition, the fig addresses conditions like ulcers, diabetes, anaemia, cancer, leprosy, liver problems, and skin disorders. Research revealed the uses of fig extracts as functional food ingredients, and clinical studies confirmed its impacts on health (George *et al.,* 2023).

FC belongs to the plant family Moraceae.. It's a fruit that goes by the name "fig." The genome of FC is made up of several gene groups. Depending on the plant component and extraction solvent, different FC-derived products (FCDP) have varying amounts of phenols, flavonoids, ortho-diphenols, proanthocyanidins, flavonols, and ascorbic acid. For instance, High amounts of phenols and flavonoids are present in the methanolic FC latex extract, as indicated by the ratio of total phenolic content (TPC) to total flavonoid content (TFC), which is frequently near to 4 (Abdel-Aty *et al.*, 2019). Numerous pharmacological characteristics, Each of the above stated phytochemicals has been associated with various benefits, such as anti-oxidative, anti-apoptotic, anti-microbial, anti-tumorigenic, and anti-inflammatory properties (Abdel-Aty et al., 2019 & Ali et al., 2012).

FC active compounds with a high potential to bind various molecular targets have been found through in silico investigations (Gurung *et al.*, 2021 & Mansoor *et al.*, 2023). The effects of FCDP, which are centered on reversing the impairments to the dysregulation of oxidative stress, inflammation, cell cycle, glucose and lipid metabolism, have been experimentally evaluated and supported by in vivo and in vitro research. These effects are condition-, disease-, and cell-specific rather than generic. For instance, FC mostly promotes cell death in tumoral tissues (Morovati *et al.*, 2022), while at the same doses, FC frequently has no harmful effects on cells that have grown properly. Moreover, FC might help stem cells maintain their viability and regulate their stemness behavior. The proliferation and maintenance of germ line stem cells treated with FC were seen in (Makoolati *et al.*,2022). Therefore, FC is advantageous for use in natural applications or for use by pharmacologists and bioengineers to synthesise products such as polymer scaffolds and nanoparticles (Shahzad Shirazi *et al.*, 2022 ; Jacob *et al.*, 2017 ; El-Sayed *et al.*, 2019).

This study looked at how dried fig and pomegranate molasses might lessen the hepatotoxicity of carbon tetrachloride in rats by analyzing liver function, kidney function, blood count, and weight of rats with hepatocellular toxicity before and after treatment with pomegranate molasses and fig.

Materials and Methods

Materials:

Rats and Diet:

Researchers bought male Sprague Dawley albino rats weighing 170±10g from Cairo University's Faculty of Veterinary Medicine's animal laboratory in which the experiment was carried out.

Components of the basic diet were purchased from El-Gomhorya Company in Cairo, Egypt.

The institutional Animal Care and Use Committee (ARC-IACUC) Agricultural Research Center, has approved the protocol. The recognized number of Cairo University (ARC) is HU 78 24.

Chemicals and Fed Ingredient:

Kits for measuring liver and kidney function with carbon tetrachloride (CCl_4) were acquired from Sigma-Aldrich Co. (St. Louis, Missouri, USA). We bought the pomegranate from the nearby market.

Methods:

Pomegranate Molasses (PM) and Fig (F) Preparation:

Pomegranate fruits were bought from the local market, cleaned, skinned, and crushed. The fluid was then extracted, filtered, and concentrated using slow heating in an open vessel until the refractometer read 75% of the total soluble solids (Al-Marazeeq *et al.,* 2017)

While fig dried on solar energy to turn it into powder

Preparation of Basal Diet:

Reeves et al.'s (1993) instructions were followed in preparing the basal diet (AIN-93M). The diet was designed to provide rats with the appropriate amounts of nutrients. PM or fig driyed powder was added at levels of 2.5%, 5% and 10% (on the diet).

All study groups consume diets that are composed of 14% protein, 4% salt mixture, 1% vitamin mixture, and 10% fat.

Carbon Tetrachloride (CCl_4):

Carbon tetrachloride (CCl_4) as a 10% liquid solution. Used as toxic material for hepatic poisoning in accordance with Passmore & Eastwood(1986).

Induction of Hepatotoxicity

The method of CCl_4-induced hepatotoxicity that we employed in this investigation (10 ml/kg of a 1:1 v/v combination of CCl_4 and olive oil) was previously reported by Li et al. (2015).

Determination of Total Phenols and Total Flavonoid:

Total phenolic and total flavonoid content concentrations were calculated using the method of (Zilic et al., 2012) and expressed as mg of gallic acid equivalent (GAE) and catechin equivalent (CE) per 100 g FW of sample, respectively.

The modified approach of Shimada et al., (1992) was utilized to measure the 1,1-diphenyl-2-picrylhydrazil (DPPH) radical scavenging activity.

Experimental Design:

The basal diet was supplied to forty eight male albino rats, and they had unlimited access to water. Prior to beginning the experiment for acclimatization, animals were kept for one week at typical conditions of humidity (50–60%), temperature (20–25°C), and light (12 hours of light and 12 hours of darkness). Pomegranate molasses or dried fig (25, 50, 100) gm for each percentage was solved in 1000 ml distilled water to 10 min to obtain (2.5%, 5%, 10 %) solvent respectively. Then, refrigerated the solvent for the experiment. Rats were divided into eight groups of six animals each as follows:

Group 1: (N = 6) fed on a Basel diet and used as a negative control (Negative control).

Group 2: Fed on a Basel diet, injected with CCl_4 according to the above protocol and used as hepatotoxicity control.

Group 3: Fed on a Basel diet with injected CCl_4 as above and administered (1ml/ kg/rat) daily from 2.5 % pomegranate molasses solvent for 6 weeks.

Group 4: Fed on a Basel diet, injected with CCl_4 as above and administered (1ml/ kg/rat) daily from 5 % pomegranate molasses, for 6 weeks.

Group 5: Fed on a Basel diet, injected with CCl_4 as above and administered (1ml/ kg/rat) daily from 10 % pomegranate molasses, for 6 weeks.

Group 6: Fed on a Basel diet suplemented with 2.5 % dried fig powder and injected CCl_4 as above for 6 weeks.

Group 7: Fed on a Basel diet suplemented with 5 % dried fig powder and injected with CCl_4 as above for 6 weeks.

Group 8: Fed on a Basel diet suplemented with10 % dried fig powder and injected CCl_4 as above for 6 weeks.

In the first six weeks of the experiment, animals of groups (2), (3), (4),(5),(6),(7) and (8) followed the above protocol to induce

hepatotoxicity in rats. At the end of experiment after ~6 weeks of the experiment, rats were sacrificed after overnight fasting. Samples of blood were drawn from the portal vein as much as feasible and placed in dry, clean tubes to clot at room temperature (26–27 ºC). After centrifuging the blood samples for 15 minutes at 3000 rpm, the serum was carefully separated and stored at -20°C until analysis.

Biochemical Analyses

Serum Analysis:

Blood was drawn, and liver function tests (ALT, AST) were performed using serum from non-heparinized blood. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in serum using the Young (2001) technique.

Kidney Functions Parameters:

Serum alkaline phosphatase (ALP) was done based on the procedure outlined by Wenger et al., (1984).The procedure outlined was used to determine the serum total protein concentration by Burtis and Ashwood (1999). Blood urea nitrogen was calculated using the procedure outlined in (Fossati et al., 1980). The serum level of creatinine was determined as the principle as stated by Delanghe and Speeckaert, (2011).

DPPH Radical-Scavenging Activity:

For every sample, 0.1 g was produced in 50 milliliters of methanol. A portion of the extract (100 µl, 0.2 mM) was combined with a methanol-dissolved DPPH radical. After stirring, the mixture was allowed to stand in the dark for fifteen minutes. Next, the absorbance was measured in relation to a blank at 517 nm. The formula for calculating the % scavenging effect was [(A0 – A1) / A0] × 100. Thus, according to Brand-Williams et al. (1995), A0 represents the absorbance of the control (without sample) and A1 represents the absorbance when the sample is present.

Hematological Parameters:

Hema Screen 18 automated hematology analyzer (Hospitex Diagnostics, Sesto Fiorentino, Italy) was used to measure the following parameters in heparinized blood: total leukocyte count, relative differential leukocyte count including neutrophils and lymphocytes, platelet count, hemoglobin concentration (Hb), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), packed cell volume (hematocrit, PCV), mean cell volume (MCV), red cell distribution width-coefficient of variation (RDW-CV), hemoglobin concentration (Hb), mean corpuscular hemoglobin

concentration (MCHC), mean corpuscular hemoglobin concentration (MCHC), hemoglobin concentration (Hb), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC).

High-Performance Liquid Chromatography (HPLC) of Phenols in(PM and fig):

Equipped with a quaternary pump, a Kinetex® 5µm EVO C18 100 mm x 4.6 mm (Phenomenex, USA), the Agilent 1260 Infinity HPLC Series (Agilent, USA) operates at 30oC. A ternary linear elution gradient with (A) HPLC grade water 0.2% H3PO4 (v/v), (B) methanol, and (C) acetonitrile is used to produce the separation. A 20 µL injection volume was used. Detection: VWD detector, as specified (Agilent Application, 2014), set at 284 nm.

Histopathological Examination:

Tissue samples of liver were removed With great care, the sample was fixed in 10% neutral buffered formalin, dehydrated in increasing alcohol grades, cleaned, embedded in paraffin, sectioned at 5 µm thickness, stained with H&E, and inspected under a microscope (Bancroft *et al.*,1996).

Statistical Analysis

Using the statistical software for social science (SPSS) version 16, a one-way analysis of variance (ANOVA) and the Duncan Test were used to compare all treatment groups. The data were presented as mean ± STD, with $p<0.05$ indicating significance(Bailey, 1995).

Results and Discussion

Serum Analysis:

Kidney functions (urea ,creatinine) and total protein in rats from six weeks of experimentation among the various groups. presented in Table1.

Table 1, presented that serum levels of creatinine of the treated rat did not significantly differ compared to the normal control rats (negative control group), except Urea that was Significantly higher values of urea were noticed for positive and negative control than those of the other experimental groups. Administration of (PM) has a protective effect against CCL_4-induced some blood parameters elevation.

Table (1): Renal Functions (Creatinine, Urea, and Total Protein) in Rats fed on Pomegranate Molasses(PM) and Fig(F) of the different experimental groups after 6 Wk.

Groups / Parameters	Urea mg /dl	Creatinine mg /dl	T.P (g/dl)
Negative Control	44.00^{c} ±1.00	0.85^{a} ±0.05	5.00^{b} ±0.10
positive Control	68.50^{a} ±2.00	1.00^{a} ±0.10	5.65^{a} ±0.15
2.5%(PM)	68.00^{a} ±2.50	1.20^{a} ±0.20	5.45^{ab}±0.25
5%(PM)	65.50^{b} ±1.00	1.00^{a} ±0.00	5.25^{ab}±0.15
10%(PM)	57.00^{b} ±0.50	0.90^{a} ±0.10	5.25^{ab} ±0.15
2.5% F	68.60^{a}±1.28	1.06^{a} ±0.04	5.62^{a} ±0.12
5% F	65.00^{b} ±4.04	0.92^{ab}±0.04	5.20^{ab} ±0.07
10% F	64.60^{b} ±2.20	0.82^{b} ±0.07	5.3 ±0.33

Mean values in each raw having different superscript (a, b, c) denote significant Difference, Urea : (N: 15 – 45 mg/dl) Creat.: (N: 0.5 – 1.5 mg/dl) T.P : (N: 6 – 8 g/dl)

Table 1 also shows that treatment with CCL_4 resulted in a significant increase in total protein (T.P) compared to the negative control group.

However, CCL_4 therapy resulted in a significant reduction in total protein levels when compared to the negative control group. Rats treated with pomegranate peel at all levels showed a substantial decrease in total protein, values when compared to the positive control group. Pomegranate peel significantly increased blood albumin levels at all levels, with no significant difference between the 5% and 10% levels compared to the negative control group. Total protein levels increased significantly in contrast to the group under positive control.On the other hand, the creatinine level showed a lower concentration than the negative control at a concentration of 10%, the fig concentration was at a level of 2.5% in total protein, which was near to the positive control. In contrast to the negative control, the level of urea was 2.5%, which was the highest percentage.

In this respect , Shimaa (2022) shows the effect of three amounts of pomegranate peel powder on CCL_4-induced toxicity in rats. Normal control rats had serum urea nitrogen, creatinine, and uric acid values of 2.77±0.25, 20.1±1.23, and 0.41±0.02 mg/dL, in that order. Serum levels of creatinine, urea nitrogen, and uric acid were measured in the positive group (+ve)increased considerably to 3.87±0.40, 50.87, and 0.71 mg/dl, respectively ($p < 0.05$). Rats treated with POP had considerably lower levels relative to the positive control group in terms of uric acid, creatinine and urea. Group (5) treated with 6% POP showed steady improvement in renal function.

Rats with CisPt-induced AKI have considerably higher serum urea, creatinine, Hcy and kidney Hyp, lipid peroxidation, kidney GSH, NO, and TAC, while their control rats have significantly lower kidney GSH, NO, and TAC. After being treated with Au-NPs and fig extract, renal function improved with an effective ROS scavenging capacity, especially at a ratio of (3:2) Dogara, et al ., (2024)

According to Kumar et al. (2018), Compared to the thioacetamide-treated group, the group treated with Ficus Bengalensis Latex at a dose of 300 mg/kg b.w. and thioacetamide at a dose of 100 mg/kg b.w. exhibited a higher level of total protein (6.12±0.11).

Table 2 demonstrated the effect of (PM) and fig on liver enzymes (ALT, AST and Alb) (U/L) in rats of the different experimental groups.

The results, as recorded in the Table 2, showed that thre were no significant differences in ALT between the positive and negative control, while there were significant decreases in both 10 and 5%(PM) groups compared to the control group. On the other hand, there were no significant differences in AST of the different experimental groups, except the last group which contain 10 % PM and 10 % F(45 ,44.20 u/l) respectivily.

Table (2) shows that treatment with CCL_4 resulted in a significant increase in the activity of alkaline phosphatase (AlP), compared to the negative control group. However, CCL_4 therapy resulted in a significant reduction in serum albumin levels when compared to the negative control group. Rats treated with pomegranate peel at all levels showed a substantial decrease in ALP, values when compared to the positive control group. Pomegranate peel significantly increased blood albumin levels at all levels, with no significant difference between the 5% and 10% levels compared to the negative control group. (AlP) levels increased significantly as compared to the positive control group.

Table (2): ALT , AST and Alb in rats fed on Pomegranate Molasses (PM) and Fig(F) of the different experimental groups after 6 wk.

Groups / Parameters	ALT (GPT) u/l	AST (GOT) u/l	Alb (mg/dl)
Negative Control	44.50^{ab} ±0.5	42.50^{ab} ±2.5	3.40^{ab} ±0.10
positive Control	51.50^{a} ±2.5	51.00^{a} ±6.0	3.85^{a} ±0.05
2.5%(PM)	48.50^{ab} ±3.5	49.00^{ab}±0.00	3.70^{a} ±0.10
5%(PM)	47.00^{b} ±1.0	47.50^{ab} ±1.5	3.55^{a} ±0.25
10%(PM)	45.50^{b} ±0.00	45.00^{b} ±2.0	3.05^{b} ±0.05
2.5%F	48.60^{a} ±5.98	48.00^{ab} ±2.92	3.56^{ab} ±0.07
5% F	46.00^{a} ±1.94	47.20^{ab} ±1.58	3.82^{a} ±0.06
10% F	45.00^{b} ±3.34	44.20^{b} ±1.65	3.24^{b} ±0.30

Mean values in each raw having different superscript (a, b, c) denote significant difference
ALT : (N: up to 40 U/L) AST : (N: up to 40 U/L) AST: Aspartate transaminase
ALT: Alanine transaminase

This finding are in agreement with Shimaa (2022) who showed that rats treated with pomegranate peel had significantly lower levels of ALP, total bilirubin, ALT, GGT, and AST compared to the control group. Regarding serum albumin levels, pomegranate peel at all levels, there was significant elevation, with no significant difference between the negative control group and the pomegranate peel groups at 4% and 6%. Total protein and globulin levels increased significantly compared to the positive control group.

Ali et al. (2021) found that CCl_4 intoxication significantly increased serum ALP, ALT, and AST levels compared to the control group. However, pomegranate peel (PPE) and its fractions significantly reduced these levels, indicating repair of hepatocyte destruction and preservation of cell membrane and hepatic architecture.

According to Foaud et al. (2018), CCL_4 treatment significantly increased serum ALT, AST, and AlP levels, whereas serum total protein levels remained unchanged. Melo et al. (2015) found that rats with CCl_4 hepatic damage had higher levels of ALT and AST compared to the negative control group. El-Hadary and Hassanien (2016) found that CCl_4 treatment significantly increased the activity of ALP, ALT, and AST enzymes compared to the negative control group. However, protein parameters (A/G ratio, globulin, albumin, and total protein) decreased overall. According to Abdel-Rahman & Abd El-Megeid (2006) and Hanaa (2014), administering CCl_4 caused liver damage in mice, as measured by blood plasma AlP, AST, bilirubin, and ALT levels. However, POP therapy significantly improved these alterations.

The infected animal group showed higher levels of ALT, AST, and ALP compared to the control group. Pre-treatment with Ficus extract and PZQ showed the strongest hepatoprotective effect El-Shabasy, *et al.*, (2022).

Biochemical testing showed increased serum enzyme levels in the rifampicin-treated group in contrast to the group under authority, indicating liver injury. The group treated with Ficus carica extract showed a considerable reduction in SGPT and SGOT levels Gond & Khadabadi (2008)

Serum levels of ALP (132.6±8.40) were found to be lower in the groups treated with thioacetamide (dosage 100 mg/kg b.w.) and Ficus Bengalensis Latex (dose 300 mg/kg b.w.) than in the group treated with thioacetamide (182.4±5.20). Ficus Bengalenesis Latex was found to be less effective than Carica Papaya Latex.The hepatoprotective

examination revealed that both latex significantly decreased the elevated blood parameter and boosted the rat hepatotoxin-induced drop in total protein level Kumar and others (2018).

Total flavonoid ,Total phenols and DPPH of PM and fig are shown in Table 3 .

Phenolic and flavonoid molecules are the most antioxidants, preventing oxidative cell damage and acting as anti-inflammatory, anti-thrombotic, and anti-allergic agents (Uchegbu *et al.,* 2016). The total flavonoid, total phenol, and DPPH levels in the Pomegranate powder plant(PM) and fig were determined and shown in Table 3.

The total flavonoid and total phenol, levels in the Pomegranate powder plant(PM) and fig were 35.29, 23.64 and 41.65, 18.96 ml / 100 mlgm ,respectively. Table (3) showed DPPH radical scavenging activity (%)

Table (3): Total flavonoid ,Total phenols and DPPH of Pomegranate Molasses (PM) and Fig (F)

Groups / Parameters	**(PM)**	**Fig**
Total flavonoid ml / 100 mg	35.29	41.65
Total phenols ml / 100 mg	23.64	18.96
DPPH%	63.21	59.83

concentration of the Pomegranate powder plant(PM) and fig it was recorded 63.21 and 59.83% ,respectively.

In this respect, Bakır *et al.,* (2015) discovered that the pomegranate extract's total phenolic content was 26.25 mg GAE/0.5 ml, while the amount of flavonoids was 31.50 mg/0.5 ml. A research by MI et al. (2000) shows the amount of phenolic compounds in pomegranate extract is double that of green tea (1029 mg/L).

Dogara, et al ., (2024) reported that highest amounts of DPPH (41.6%) and FRAP (8504 mg FeSO4/kg DM) were found in fig seeds extracted with 50% (v/v) aqueous methanol (30). The DPPH experiment revealed that fresh figs had the highest levels of antioxidant activity.

Initial weight (g), final weight (g) and BWG (g) in rats of the different experimental groups after 6 weeks.were presented in Table 4.

All groups had a lower body weight but CCl_4 group rats had reduced more than a quarter of the mean body weight in contrast to the control group's. The animals fed with (2.5%, 5% PM) had a similar lower mean weight (23.5% of initial weight) while animals fed with (10% PM) had a

lower mean weight only (21% of Initial weight) which indicated that supplementation of PM along with CCl_4 resume the lost weight caused by CCl_4 as compared to that of control group.

Meanwhile, the CCL_4-treated group recorded the lowest body weight gain, as compared with all groups these may be due to loss of appetite and the acute hepatic damage spotted in rats intoxicated using CCl_4.

These outcomes are consistent with Shimaa (2022) who claimed that mice in the CCl_4 treatment group had lower body weights than the control group. It was discovered that rats given with pomegranate peel powder (POP) at 2%, 4%, and 6% had a substantial increase and protective effect in all nutritional parameters in contrast to the positive control group. Conversely, though POP at a level of 6% had the best protective potential against CCl_4 poisoning.

Table (4): Initial Weight (g), Final Weight (g) and BWG (g) in rats fed on Pomegranate Molasses(PM) and Fig(F) of the different experimental groups after 6 wk.

Groups / Parameters	Initial weight (g)	Final weight (g)	BWG (g)
Negative Control	183.25^{a} ±22.29	139.50^{a} ± 28.54	-43.75^{b} ±11.44
positive Control	184.25^{a} ± 32.99	135.00^{a} ±17.64	-49.250^{ab}±0.18
2.5%(PM)	184.00^{a} ±27.77	140.75^{a} ± 16.11	-43.25^{ab} ±6.39
5%(PM)	163.00^{a} ±21.78	124.75^{a} ± 18.57	-38.25^{a}± 9.39
10%(PM)	174.50^{a} ± 24.44	137.75^{a} ±32.58	-36.75^{ab}±5.61
2.5%F	200.20^{a} ±6.34	145.80^{a} ±10.05	-54.40^{a}±5.59
5% F	186.20^{a} ±19.84	142.0^{a} ±9.02	-44.20^{ab}±12.21
10% F	197.60^{a} ±33.88	161.0^{a} ±21.15	-36.6^{b} ±14.90

Mean values in each raw having different superscript (a, b, c) denote significant difference

Nemiche et al (2022) found that after one month of treatment with nickel chloride, there was no significant difference in these parameters compared to the control group. FCE administration resulted in a considerable ($p < 0.001$) increase in body weight (+24%) and gain (+91%) in the Ni + FC group, compared to the Ni group. The FC group gained significantly more body weight (+52%) than the untreated group ($p < 0.01$). Rats in the various experimental groups did not differ significantly in terms of liver weight or liver body weight ratio.

Table (5) showed Organs weight in rats of the different experimental groups after 6 wk.

Table(5) :Organs weight in rats fed on Pomegranate Molasses (PM) and Fig (F) of the different experimental groups after 6 wk.

Groups / Parameters	Liver	Kidney	Spleen	Heart
Negative Control	$3.86^{a} \pm 0.66$	$0.87^{a} \pm 0.09$	$0.46^{a} \pm 0.13$	$0.38^{a} \pm 0.11$
positive Control	$3.75^{a} \pm 0.93$	$0.81^{a} \pm 0.18$	$0.45^{a} \pm 0.06$	$0.41^{a} \pm 0.04$
2.5%(PM)	$3.39^{a} \pm 0.32$	$0.75^{a} \pm 0.05$	$0.35^{a} \pm 0.08$	$0.35^{a} \pm 0.06$
5%(PM)	$3.70^{a} \pm 1.09$	$0.76^{a} \pm 0.17$	$0.38^{a} \pm 0.09$	$0.37^{a} \pm 0.15$
10%(PM)	$3.18^{a} \pm 0.33$	$0.68^{a} \pm 0.06$	$0.36^{a} \pm 0.05$	$0.33^{a} \pm 0.07$
2.5%F	$3.73^{a} \pm 0.28$	$0.88^{a} \pm 0.04$	$0.44^{a} \pm 0.05$	$0.36^{a} \pm 0.02$
5% F	$3.84^{a} \pm 0.68$	$0.75^{a} \pm 0.01$	$0.38^{a} \pm 0.05$	$0.39^{a} \pm 0.05$
10% F	$3.92^{a} \pm 0.39$	$0.76^{a} \pm 0.14$	$0.41^{a} \pm 0.07$	$0.34^{a} \pm 0.04$

Mean values in each raw having different superscript (a, b, c) denote significant difference

There was no significant difference observed at organs weight in rats from each experimental groups after 6 wk.

In this respect, Dogara, et al ., (2024) mentioned that One of the biggest organs in the body and the primary location of metabolism and excretion is the liver. Injuries to this organ, which is crucial for eliminating toxins that are both naturally occurring and artificially introduced, can have detrimental effects on a person's health.

Effect of (PM) and fig on rats' hemoglobin, platelet count, hematocrit, MCV, MCHC%, and RBC count of the different experimental groups after 6 weeks presented in Table 6.

These changes included an increase in hemoglobin, an increase in RBC for animals fed on PM in all concentrations but hematocrit increased at high concentrations dosage of PM (5%, 10%).Which indicated that supplement of PM along with CCl_4 resume the anemic effect caused by CCl_4 as compared to that of control group.

Table 6: Effect of Pomegranate Molasses (PM) and Fig (F) on Hb, RBC count, Hematocrit, MCV, MCHC% and Platelets count of the different experimental groups after 6 wk.

Groups / Variables	Hb	RBC	Hematocrit	MCV	MCH	MCHCP	Platelets count
	g/dl	Count	(PCV)%	Fl	pg.	g/dl	Thousands /cmm
Negative Control	11.70^{a} ±0.30	4.46^{a} ±0.21	36.76^{a}±0.32	88.00^{a} ±1.00	27.00^{a} ±0.20	32.00^{a} ±1.00	394.66^{b} ±1.52
Positive Control	10.46^{c} ±0.47	3.86^{c} ±0.15	35.00 ^{b} ±1.00	84.00^{b}± 1.00	25.30^{b} ±0.61	30.30^{a} ±0.61	397.66^{a} ±1.52
2.5% (PM)	10.83^{bc} ±0.15	4.03^{bc} ±0.15	34.83^{b}±0.15	84.30^{b} ±0.60	26.00^{ab} ±1.00	31.0 ^{a} ±1.00	377.33^{d} ±1.52
5%(PM)	11.13^{ab} ±0.32	4.03^{bc} ±0.15	35.43^{b}±0.51	84.66^{b} ±1.52	27.00^{a} ±1.00	31.63^{a} ±1.18	312.00 ^{e} ±2.00
10%(PM)	11.46^{a} ±0.50	4.43a^{b} ±0.51	36.46^{a}±0.50	85.00^{b} ±1.00	27.50^{a} ±0.50	32.00^{a} ±1.00	311.66 ^{e} ±1.52
2.5%F	10.3^{c} ±0.26	3.82 ^{c} ±0.11	34. 81^{b} ±0.12	84.00^{b} ±1.00	25.30^{b} ±0.61	30. 30 ^{a} ±0.61	398. 75 ^{a}±0.36
5% F	10.40^{c} ±0.26	3.95^{c} ±0.05	34.97 ^{b}±0.14	85.00^{b} ±1.00	26.66 ^{ab} ±1.44	31.00 ^{a} ±1.00	387.29 ^{c}±0.15
10% F	10.81^{bc} ±0.25	4.04 ^{bc} ±0.14	35.26 ^{b}±0.25	87.03^{a} ±0.95	27.16^{a} ±0.9	31.21^{a} ±0.70	387.59 ^{c}±0.20

Mean values in each raw having different superscript (a, b, c) denote significant difference
The normal number of platelets 150 to 400 × 109/L. Thousands/cmm

Soliman *et al.*, (2022) mentioned that Male albino rats were used to examine the effects of pomegranate molasses ethanolic extract on anemia induced by phenylhydrazine (PHZ). After administering PHZ, red blood cells (RBCs) droppd in number, while the mean cell volume, hematocrit, and hemoglobin concentration rose. The findings for concentration, reticulocyte count, leukocyte count, platelet count, and mean corpuscular hemoglobin (MCH) were all consistent.

Yıkmış *et al.*,(2022) reported that Thermosonication treatment (TS-PJ) had better sensory qualities. (TS-PJ)led to the detection of increases in the element (Fe)The bioavailability of bioactive substances was better conserved by TS-PJ in an in vitro simulated gastrointestinal media Which indicates why hematological and other parameters improved .

SiO2NPs, Al2O3NPs, and ZnONP treatment significantly decreased Hb (36.59%, 44.38%, and 49.07%, respectively), RBC count (11.16%, 22.68%, and 38.01%), MCV (9.59%, 12.03%, and 14.34%), MCH

(28.33%, 27.79%, and 29.78%, respectively), and MCHC (20.63%, 16.20%, and 17.68%). The corresponding platelet counts are 13.66%, 15.84%, and 19.39%. The FOD-SiO2NPs, FOD-Al2O3NPs, and FOD-ZnONP-treated groups had higher Hct percentages, RBC counts, Hb concentrations, and platelet counts, but lower WBC counts, neutrophils, lymphocytes, and monocytes compared to the SiO2NPs, Al2O3NPs, and ZnONP-treated groups. The administration of FOD-SiO2NPs, FOD-Al2O3NPs, and FOD-ZnONP did not affect MCV, MCH, or MCHC levels in the blood compared to SiO2NPs, Al2O3NPs, and ZnONP. Naji *et al.*,(2023).

Effect of (PM) and fig on rat WBC count, neutrophils, basophils, lymphocytes, monocytes, and eosinophils of the different experimental group after 6 weeks presented in table 7.

From table 7 we can noted that CCl_4 group rats had a decrease in WBC, neutrophils, lymphocytes Monocytes, and Eosinophils, as compared to that of control group. Therefore, our results suggest that the intake of PM improve antioxidant activity and heaptoxicity recovery and CBC outcomes

Our findings concur with Soliman *et al.*, (2022) who found that Molasses given before PHZ reduced RBC anomalies, decreased MCH, leukocyte, platelet, and reticulocyte counts, and elevated RBC, hematocrit, and hemoglobin concentration.

Naji *et al.*,(2023) reported that SiO2NPs, Al2O3NPs, and ZnONP treatment the blood samples from the experimental group showed a noteworthy rise in the percentages of WBC (19.39%, 22.43%, and 20.92%, respectively), neutrophils (26.35%, 32.57%, and 31.01%), lymphocytes (12.65%, 12.27%, and 14.15%, respectively), and monocytes (26.63%, 30.12%, and 32.66%, respectively).The FOD-SiO2NPs, FOD-Al2O3NPs, and FOD-ZnONP-treated groups had higher Hct percentages, RBC counts, Hb concentrations, and platelet counts, but lower WBC counts, neutrophils, lymphocytes, and monocytes compared to the SiO2NPs, Al2O3NPs, and ZnONP-treated groups. The administration of FOD-SiO2NPs, FOD-Al2O3NPs, and FOD-ZnONP did not affect MCV, MCH, or MCHC levels in the blood compared to SiO2NPs, Al2O3NPs, and ZnONP.

Table 7: Effect of Pomegranate Molasses (PM) and Fig(F) on WBC count, Lymphocytes, Monocytes, Eosinophils, and Neutrophils of the different experimental groups after 6 wk.

Groups	WBC count	Neutrophils	Lymphocytes	Monocytes	Eosinophils
Negative Control	8.80 ^{ab} ±0.20	24.00^{a}±1.00	84.26 ^{a}±0.64	6.00 ^{a} ±1.00	1.70 ^{a} ±0.62
Positive Control	7.61^{d}±0.34	11.26^{d}±0.64	69.00 ^{d} ±1.00	4.10 ^{cde} ±0.36	1.20 ^{a} ±0.20
2.5% (PM)	8.86 ^{ab}±0.15	12.23^{cd}±0.49	79.00 ^{b}±1.00	4.13^{e} ±0.32	1.26 ^{a} ±0.25
5%(PM)	8.63^{bc}±0.32	13.00 ^{cd} ±1.00	82.33^{a}±1.52	4.26 ^{de} ±0.64	1.36 ^{a} ±0.55
10%(PM)	9.41^{a} ±0.52	17.00^{b}±1.00	84.00^{a}±1.00	4.16 ^{de} ±0.20	1.50^{a} ±0.50
2.5%F	7.68 ^{d} ±0.27	12.33 ^{cd} ±0.58	69.11 ^{d} ±1.01	4.29 ^{bcd} ±0.32	1.33^{a} ±0.14
5% F	8.01 ^{cd} ±0.33	14.00 ^{c} ±2.0	71.50 ^{c} ±1.80	5.07 ^{abc} ±0.89	1.24^{a}±0.02
10% F	8.53^{bc}±0.56	13.03 ^{cd} ±0.95	77.33 ^{b} ±2.08	5.29 ^{ab} ±0.65	1.47^{a}±0.19

Mean values in each raw having different superscript (a, b, c) denote significant difference

Histopathological Results

Control negative group showed There were no histological changes in the liver, and the hepatic parenchyma showed normal blood sinusoids and healthy hepatocytes (Fig. 1).

Group II (control positive group) showed diffuse liver alterations in the form of multifocal areas of degenerated and swelled hepatocytes which appeared vacuolated with eosinophilic cytoplasmic granules (Fig. 2).

Group III (low dose diet group) showed general improvement in all the examined tissues than the control positive group, liver reported improvement in the degenerated hepatocytes to become apparently normal (Fig. 3).

Group IV (high dose treated group) showed general improvement in all the examined tissues than the low dose diet group, liver reported complete regression in the degenerated hepatocytes which become fully normal and more improved than low dose diet group (Fig. 4).

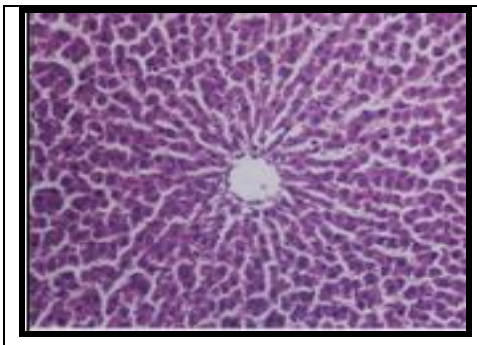

Fig. (1): Liver of negative control group, untreated rat showing the normal histology of hepatic lobule. (H and E X 200)

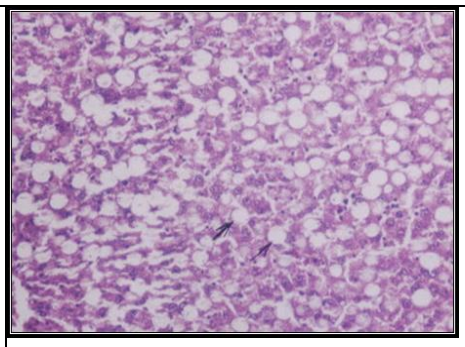

Fig. (2): Liver of rat from positive control group (CCL_4), showing vacuolar degeneration of hepatocytes with signet ring appearance of hepatocytes. (H and E X 200)

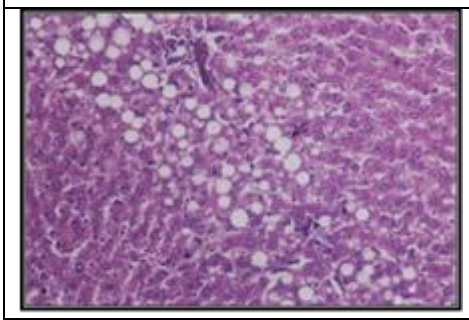

Fig. (3): Liver of rat from group (3) showing vacuolar degeneration of some hepatocytes

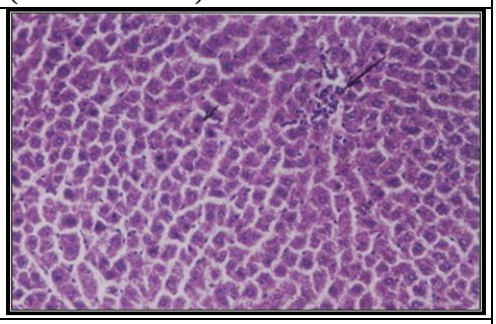

Fig. (4): Liver of rat from group (4), showing necrosis of sporadic hepatocytosis (small arrow) associated with sinusoidal heucocytosis (large arrow). (H and E X 200)

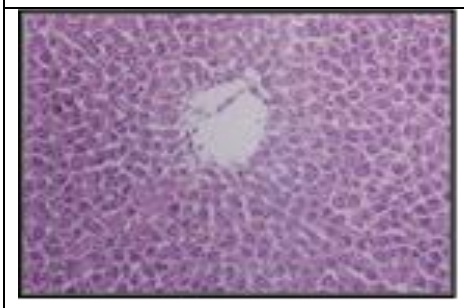

Fig. (5): Liver of rat from group (5) showing kupffer cell activation.(H and E X 200)

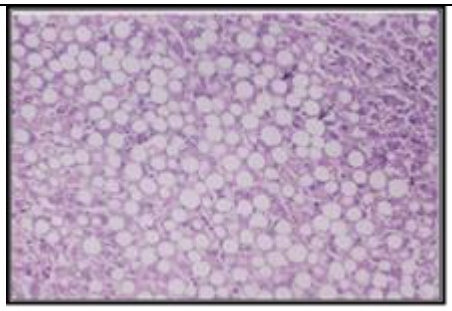

Fig. (6):Liver of rat from group (6) showing vacuolar degeneration of hepatocytes.(H and E X 200)

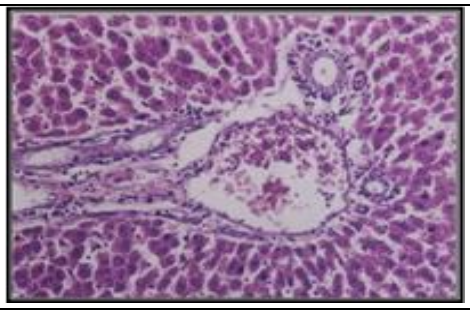

Fig. (7): Liver of rat from group (7) showing few leucocytic cells infiltrating the portal area. (H and E X 200)

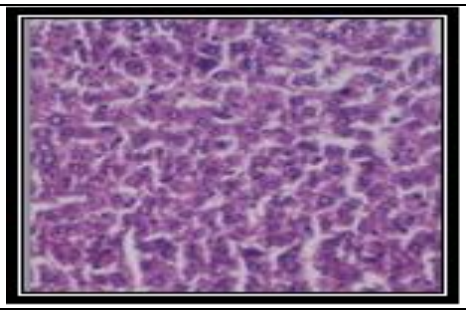

Fig. (8): Liver of rat from group (8) showing few sinusoidal leukocytosis.(H and E X 200)

Effects of date palm fruit extract (FOD) and olive oil mixed with fig fruit extract on Heart Histopathology in rats Administered with SiO2NPs, Al2O3NPs, ZnONPs over a period of 75 days. The morphological appearance of the control heart tissue was normal.Nevertheless, the NP-administered groups that received antioxidant treatment (FOD-SiO2NPs, FOD-Al2O3NPs, and FODZnONPs) showed significantly improved histopathological characteristics in the heart tissues as compared to the nonantioxidant-treated NP-administered groups (SiO2NPs, Al2O3NPs, and ZnONPs, respectively) Naji *et al.*,(2023)

In addition , NP groups treated with antioxidants showed considerably improved Comparing histopathological characteristics to non-treated NP groups. FOD treatment protects heart tissues by reducing or preventing lipid and protein oxidation through free radical scavenging.

Conclusion

As a result, our findings imply that consuming pomegranate molasses (PM) or fig improves antioxidant activity, heaptoxicity recovery, and CBC outcomes. Additionally, they can protect against CCL_4-induced blood poisoning and anemia, making it a possible natural therapy alternative. More research is needed on humans.

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التأثير العلاجي لدبس الرمان والتين على الفئران المصابة بالتسمم الكبدي برابع كلوريد الكربون

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الملخص

تمت دراسة التأثير الوقائي المحتمل لدبس الرمان والتين ضد التسمم الكبدي الناجم عن رابع كلوريد الكربون (CCl_4) في ذكور الفئران. وقد تبين أن دبس الرمان والتين يحتويان على العديد من المركبات الفينولية والفلافونويد.اجريت الدراسة على ثماني مجموعات في كل مجموعة (6) فئران؛المجموعة(1)الضابطةالسالبة(طبيعي)، في حين تم إعطاء فئران المجموعات الأخرى حقنة تحت الجلد من رابع كلوريد الكربون (CCl_4) مرتين أسبوعيًا لمدة ستة أسابيع للحث على تسمم الخلايا الكبدية. تركت المجموعة (2) كمجموعة ضابطةموجبة، بينما أعطيت باقي المجموعات دبس الرمان أوالتين المجفف بنسبة (2.5%، 5%، 10%) لمدة 6 أسابيع على التوالي. وفي نهاية التجربة تم أخذ عينات الدم للتحليل البيوكيميائي. أظهرت النتائج أن الفئران المصابة بتسمم الخلايا الكبدية لديها زيادة معنوية في مستويات AST، ALT، البروتين الكلي، الكرياتينين، ونيتروجين اليوريا في الدم، مقارنة بالمجموعة الضابطة الطبيعية. أدى إعطاء (2.5%، 5%، 10%) من الرمان والتين المجفف إلى الحفاظ على الكبدو تسبب في انخفاض كبير في مستويات AST وALT والبروتين الكلي والكرياتينين ونيتروجين يوريا في الدم بينما وكانت نسبةالزيادة في الهيموجلوبين وكريات الدم الحمراء أكثر في الفئرات التى تناولت دبس الرمان خاصة المجموعةالتى تناولته بتركيز (10%). خلصت هذه الدراسة إلى أن دبس الرمان والتين المجفف يمكن أن يكون لهما تأثير وقائي من السمية الكبدية في الفئران والتأثير على فقر الدم الناجم عن ال CCl_4، لذلك توصي هذه الدراسة بزيادة تناول دبس الرمان والتين المجفف كإجراء وقائي للمرضى الذين يعانون من أمراض الكبد والكلى.

الكلمات المفتاحية: الرمان؛ التين؛ رابع كلوريد الكربون؛ السمية الكبدية؛ إنزيمات الكبد؛ وظائف الكلى؛ الفئران.