

Effect of PMF on Hepatitis Induced by Carbon Tetrachloride in Rats

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Abstract

Nowadays liver diseases constitute a major medical problem of worldwide proportions, so it is important to search for compounds that are more effective in their therapeutic action for hepatic diseases. The objective of this study is to evaluate the protective effects of PMF derived from camel urine on hepatitis induced in Wistar male rats by carbon tetrachloride (CCL₄). Four groups of rats were treated as follows (Control, PMF orally, were intraperitoneally injected with CCL₄ and CCL₄ plus oral PMF) respectively. Physiological and histological alterations were evaluated after 7, 21, 42, 60 and 90 days. Highly significant decrease in the body weight and highly statistically increase of liver/body weight ratios were observed in rats treated with CCL₄. Moreover, the levels of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP) and total bilirubin (TBL) were statistically increased. We also observed significantly decrease in total protein (TPRT) and total albumin (TALB). Additionally, the liver sections from rats injected with CCL₄ showed liver fibrosis and necrosis, inflammatory cell aggregation and adipocyte deposition. Interestingly, the findings of this experimental study suggest that PMF may work as hepatoprotective agents against CCL₄ induced hepatic damage by reducing the physiological and histological alterations. These results also suggest that the protective effect of PMF may be attributed to their antioxidant activities.

Keywords: Hepatitis; PMF; camel urine; CCL.

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1. Introduction

Liver cells can be evoked by viral infection, toxic industrial chemicals, food additives, alcohol, fungal toxins, air and water pollutants [1]. Liver injury is characterized by either necrotic or apoptotic cell death as well as, more often, by a combination of the two. It is well known that the same stimulus can induce both types of irreversible cell death [2]. Hepatitis is an inflammation of the liver, the condition can be self-limiting or can progress to fibrosis, cirrhosis or liver cancer. Fibrosis occurs as a result of initial liver injury, including hepatocytes damage, kupffer cells activation, hepatic stellate cells (HSC) activation and proliferation [3]. Liver fibrosis is characterized by HSC proliferation and differentiation to myofibroblast-like cells, which deposit extracellular matrix (ECM) and collagen [4].

PMF is a bio-active fraction which has been extracted from camel urine [5]. Several studies carried out on identified PMF as a potential anticancer agent[5,6,7]. The effect of PMF was tested on the growth of cancer and normal cell lines used as model of human lung cancer (A549) and human fore skin (HFS). Cells were counted by two method, counter coulter and hemocytometer using trypan plue dye. In their results, PMF was found to act selectively on cancer cells while does not affect normal cells [8].

The metabolic activity and cells proliferation colorimetrically was measured by MTT assay in different cancer cell lines treated with PMF [7]. Apoptotic cell death was also determined by the TUNNEL method. Their results showed that PMF have anticancer effect by decrease in cancer cells proliferation and altering cellular metabolic activities. PMF was also tested on breast cancer cells [7], the study revealed that PMF inhibited the proliferation of MCF-7 cells in a dose dependent manner. The author suggested that PMF may help the cancer cells revert back to normal cells by enhancing oxygen transport [7]. Although, it has still not been clearly demonstrated which mechanisms are responsible for apoptosis in PMF treated tumor cell lines.

Moreover, PMF demonstrated a high cytotoxic activity against human hepatocellular liver carcinoma, human lung cancer, glioma cells, colorectal cancer cells, mice leukemia cells, skin cancer cells and breast carcinoma [7,8,9,10,11]. PMF was also found to be effective in preventing metastasis in spreading effect of leukemia cells in animal models [12].

It is well known that anticancer agents works as antiviral [13,14].There is one available published data concerning PMF hepatoprotective activity [15]. However, its anti- cancer activity on hepatocellular carcinoma was reported by several studies which showed that PMF demonstrated a high cytotoxic effect against hepatocellular carcinoma [5,7].

Under all experimental conditions used, PMF has been established to be non-toxic, and has no significant adverse effects on animals organs [16]. In addition, phase I clinical trials using capsules and syrup containing PMF have been carried out on healthy volunteers and confirm that PMF has no side effects where used as medication[17].

Moreover, high doses of PMF did not show any effect on the blood components, and liver enzymes (ALT, AST, ALP, ALB, TBIL and GGT) [18]. Abnormalities in liver function have prognostic importance in chronic heart

failure [19], whereas mice treated with PMF have no alterations in liver enzymes activity and inturn revealed the safety of PMF on heart function [18]. Moreover, biochemical results showed insignificant alteration on kidney functions including Na, K, Cl, creatinine, blood urea nitrogen, uric acid, phosphorus and total protein values in mice receiving oral PMF at various doses compared to control groups [18].

The histopathology studies of vital organs (liver and kidney) from animals administrated by therapeutic dose of PMF showed no recognizable effect on the tissues structure of studied organs. These results pointed that PMF is a safe substances even with high doses or with long administration period [18]. In our study we will investigate the effects of PMF on hepatitis induced by CCL₄ in rats.

2. Material and methods

2.1. Preparation of PMF

PMF was extracted from camel urine according to [5], the dose of PMF determined according to previous study [11]. PMF (powder) were collected in sterile bottles and kept at 4°C for not more than 2 weeks.

2.2. Induction of Hepatotoxicity by CCL₄

Hepatitis was induced by the intraperitoneal injection of CCL₄ (1ml/kg of the body weight) with paraffin oil 1:1 dilution, in the first two days of the experiments according to the method of [20].

2.3. Animals

Male albino rats of wistar strain (*Rattus norvegicus*), weighing 200- 230 g were used in the present study. The animals were obtained from the Experimental Animals Unit at King Fahd Medical Research Center, King Abdul-Aziz University, Jeddah, Saudi Arabia. Rats were acclimatized to the laboratory conditions for 10 days prior to the initiation of experimental work. The animals were housed in standard plastic cages and maintained under controlled laboratory conditions of humidity (65%), temperature (20 ± 1°C) and 12:12h light: dark cycle. Rats were fed of normal commercial chow with free access to water. The experiment was conducted in accordance to ethical guidelines of animal care ethical committee at King Abdulaziz University.

2.4. Experimental design

A total of eighty rats were randomly divided into four experimental groups, twenty rats each. The groups were divided as follows:

G1: Control rats fed only with diet and tap water.

G2: Rats which were administrated orally with PMF (1.8 g/kg/body weight).

G3: This group that was contain infected group with 1ml/kg/body weight of CCL₄, 1: 1 diluted with paraffin oil and was injected via IP routes in the first two days of the experiment.

G4: Rats that injected IP in the first two days of the experiment with 1ml/kg/body weight of CCl₄, 1:1 diluted with paraffin oil and after one week treated with Oral PMF (1.8 g/kg).

2.5. Body Weight Determinations

The body weights of the rats were determined before and after the end of the experiments using a digital balance.

2.6. Blood and tissue Collection

Five experimental animals for each group were sacrificed at 7, 21, 42, 60; 90 days, before this step the animals were not given any food for 16 hours, water was not restricted. Animals were then anesthetized with diethyl ether. Blood were collected from all animals before dissection from the retro orbital venous sinuses in inner canthus of the eye. After blood sampling, the liver was removed by careful dissection, then washed with cold 0.9 % sodium chloride saline solution and dried gently between two filter papers then weighed. Liver weight / body weight ratio was calculated for each rat by the following equation

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

2.7. Serum Biochemical Analysis

Liver function was performed for each of: aspartate transaminase (AST), alanine aminotransferase (ALT), Alkaline phosphatase (ALP), Gamma glutamyl transferase (GGT), total bilirubin (TBL), total protein (TPRT), total albumin (TALB) using spectrophotometric techniques (Dimension vista System, Siemens Healthcare Diagnostics Inc., USA). The method of [21] was used to determine the levels of serum ALT and AST. Serum GGT level was measured according to the method of [22]. The method of [23] was carried out to determine the level of Total protein. Total bilirubin and albumin concentration was determined using the method of [24].

2.8. Histopathological examinations

The liver was excised from the animals. Sections of 5 µm thickness were prepared blocks using a microtome [25]. These were processed in alcohol-xylene series and were stained with haematoxylin and eosin stain (H and E) [26]. Moreover, liver sections were subjected to Masson's trichrome stain [27]. All liver sections were examined using a light microscope and photographed.

2.9. Statistical analysis

Statistical Science for Social Package (SPSS version 20, SPSS Inc., Chicago, IL, USA) was used for data analysis, data were expressed as mean +/- SD. One way ANOVA test LSD (Least square difference test) was used for comparison between different groups and paired Student's t-test was used to compare parameters from the same animal. Graphs were made using Prism software version 5. For all tests, P- values of less than 0.05 (P < 0.05) were considered as significant [28].

3. Results

3.1. Body weight

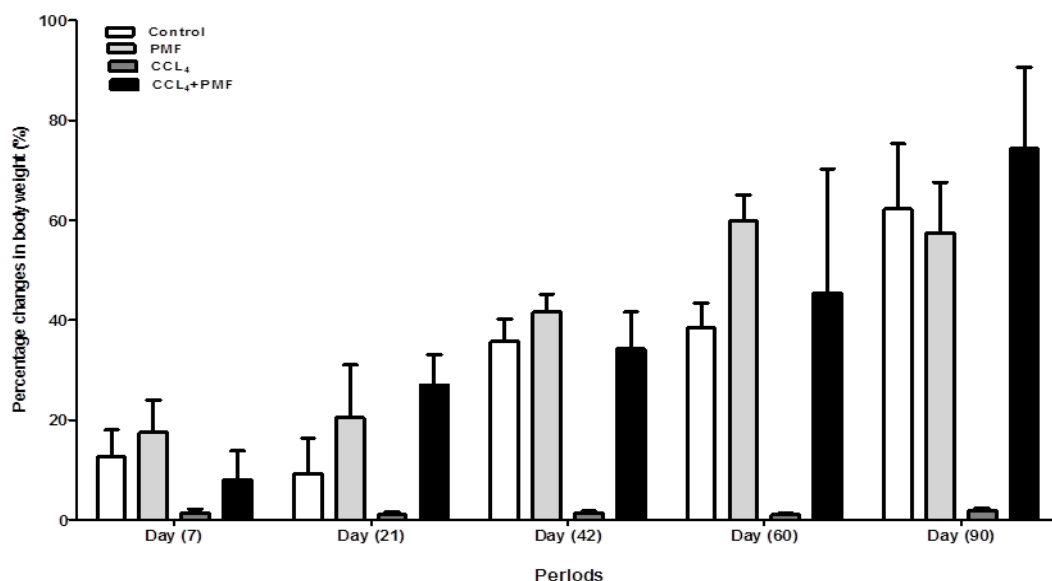


Figure 1: Changes in body weight in different groups at different periods. Data are expressed as mean \pm SE.

In this study we weight each animal before dissection, all animals showed a significant increase in the body weights in the group received oral PMF ($P < 0.05$) compared to CCL₄ group.

There was also a significant increase in body weight ($P < 0.05$) in all the animals received CCL₄+ oral PMF compared to non treated CCL₄ groups.

While there was a significant loss in the body weights in CCL₄ animal group compared to the control group at all periods ($P < 0.05$). At same time, the body weights were insignificant in animals received oral PMF at all periods compared to control group ($P > 0.05$). Moreover, all animals exposed to CCL₄ and treated with oral PMF showed no changes in the body weights during the experiments compared to control group ($P > 0.05$) (Fig. 1).

3.2. Liver weight

In the animals supplemented with oral PMF, there was a significant decrease in liver weights compared to CCL₄ group ($P < 0.05$). Compared to non treated CCL₄ group, changes in liver weights were significantly decreased in animals subjected to CCL₄+ oral PMF ($P < 0.05$). Whereas the liver weights were significantly higher in CCL₄ group compared to control group in all animals ($P < 0.05$). The changes in liver weights were insignificant in animals exposed to oral PMF when compared to negative control group ($P > 0.05$). At same time the results showed that all animals exposed to CCL₄+ oral PMF have insignificant changes in liver weights during our experiments compared to control group ($P > 0.05$) (Fig. 2).

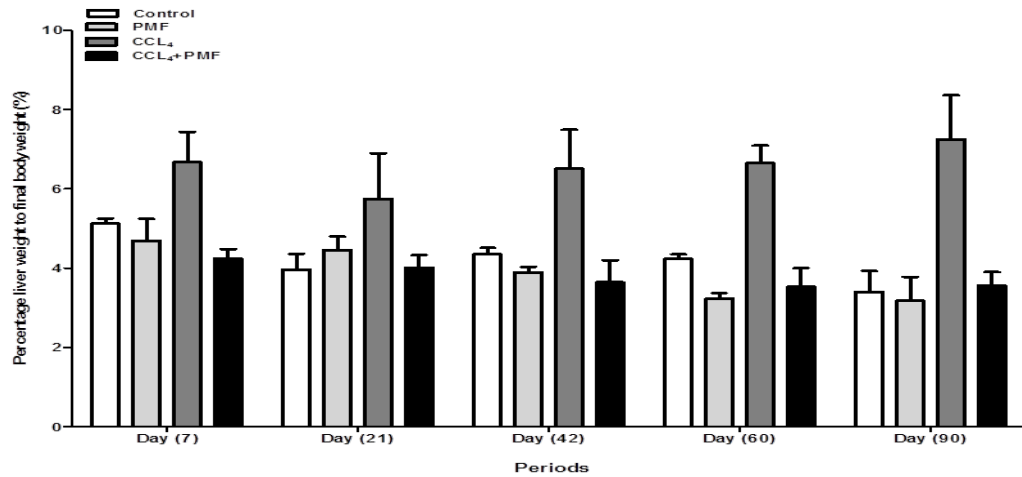


Figure 2: Changes in liver weight to final body weight in different groups at different periods. *Data are expressed as mean \pm SD*

3.3. Liver functions tests (ALT, AST, ALP, GGT and total bilirubin)

The group administered with oral PMF showed significant decrease in ALT, AST, ALP, GGT and total bilirubin levels compared to CCL₄ group at all periods ($P < 0.05$). Similar results were observed in groups treated with CCL₄ + oral PMF at all periods compared to non treated CCL₄ group ($P < 0.05$). While significant increase in ALT, AST, ALP, GGT and total bilirubin levels were observed in CCL₄ group compared to negative control group ($P < 0.05$) at all periods. The results also showed that there were insignificant changes in all tested enzyme in group exposed to oral PMF at all periods compared to control group ($P > 0.05$). In all animals exposed to CCL₄+ oral PMF the data showed insignificant changes in the same tested serum enzymes and total bilirubin levels compared to control group ($P > 0.05$) (Fig. 3, 4, 5, 6 and 7).

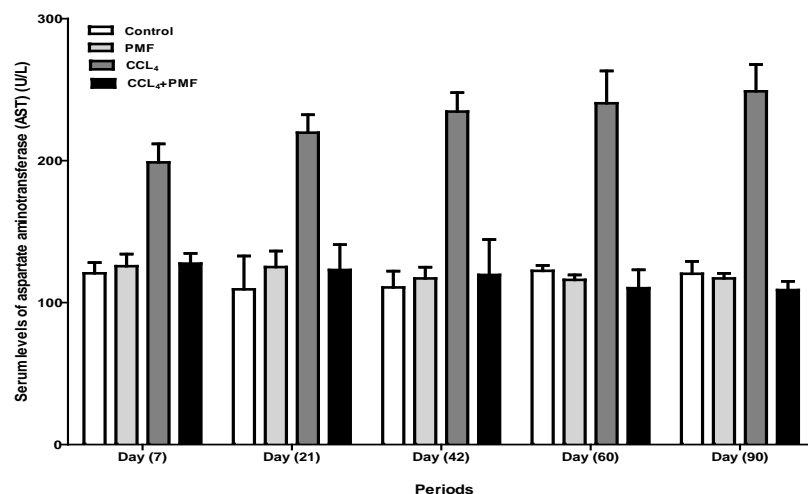


Figure 3: Changes in aspartate aminotransferase (AST) (U/L) in different groups at different periods. *Data are expressed as mean \pm SD.*

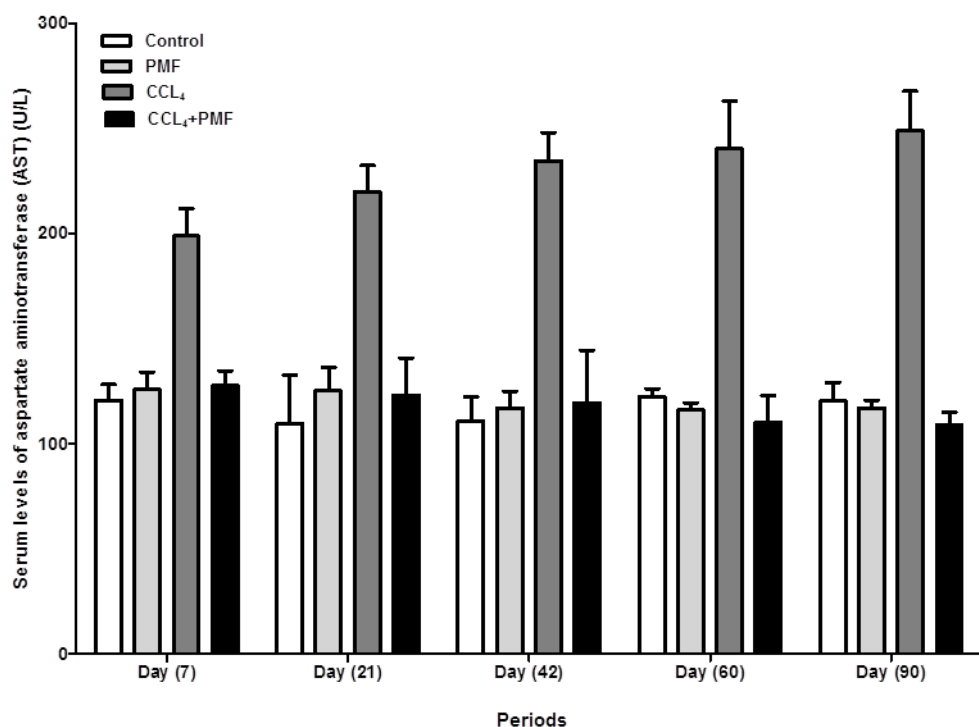


Figure 4: Changes in alkaline phosphatase (ALP) (U/L) in different groups at different periods. Data are expressed as mean \pm SD.

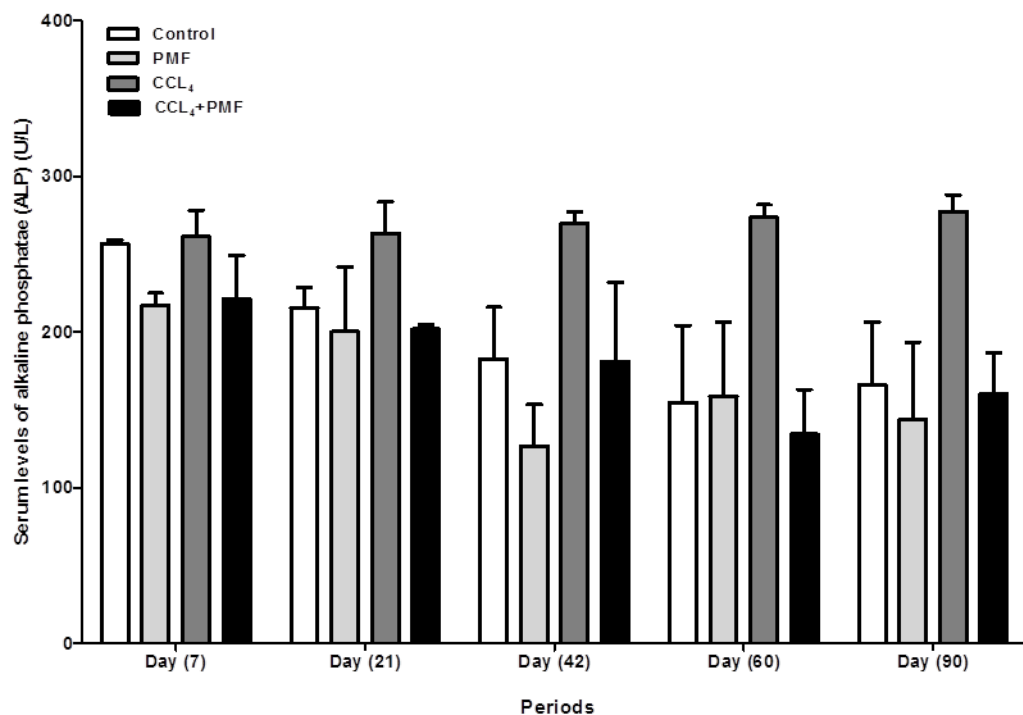


Figure 5: Changes in gamma-glutamyl transferase (GGT) (U/L) in different groups at different periods. Data are expressed as mean \pm SD.

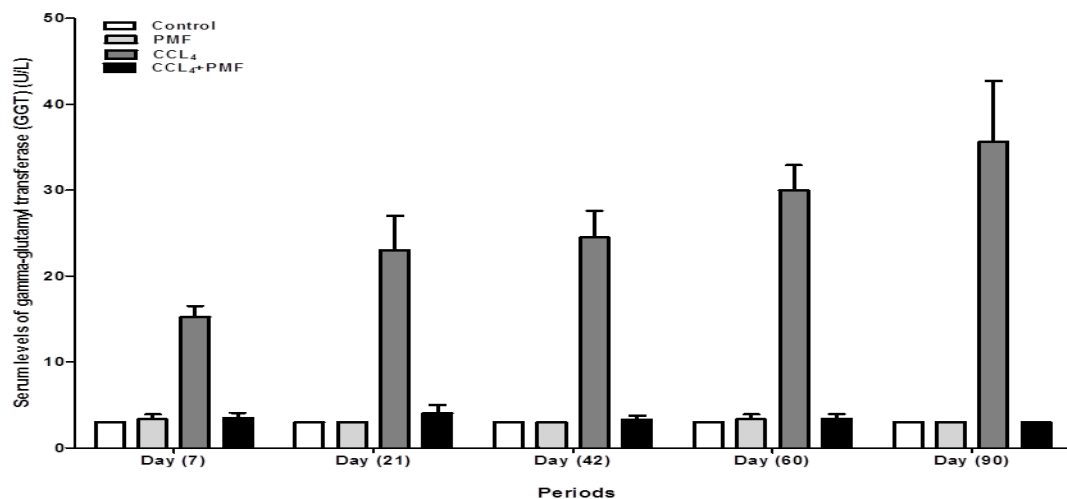


Figure 6: Changes in serum levels of total bilirubin (TBIL) (umol/L) in different groups at different periods. Data are expressed as mean \pm SD.

3.4. Total protein and albumin

All animals supplemented with oral PMF showed significant increase in total serum protein and albumin levels at all periods compared to CCL₄ group ($P < 0.05$). The results also showed significant increase in serum total protein and albumin levels in animals injected with CCL₄ then treated with oral PMF at all periods compared to non treated CCL₄ animals ($P < 0.05$). The total protein and albumin levels of the serum in CCL₄ group were significantly lower compared to control group CCL₄ group ($P < 0.05$). Whereas, compared to control group there was insignificant changes in total serum protein and albumin levels in animals received oral PMF and the animals received to CCL₄+ oral PMF ($P > 0.05$) (Fig. 8 and 9).

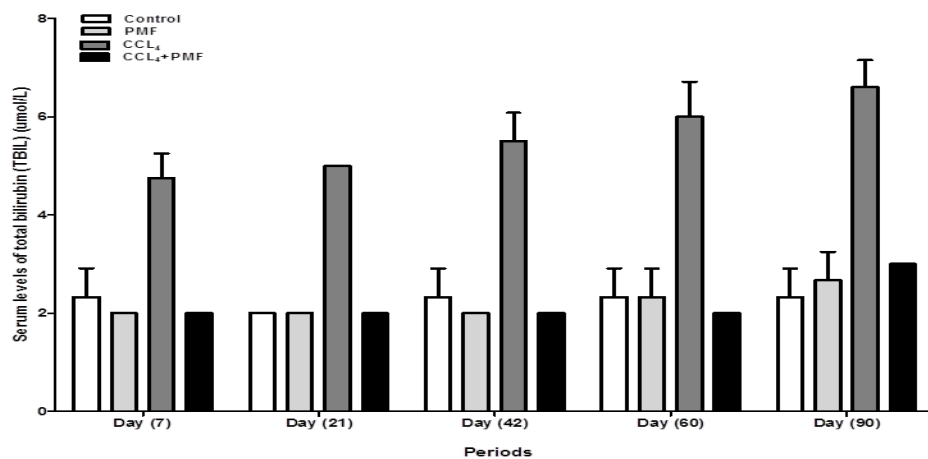


Figure 7: Changes in serum levels of total protein (TP) (g/L) in different groups at different periods. Data are expressed as mean \pm SD.

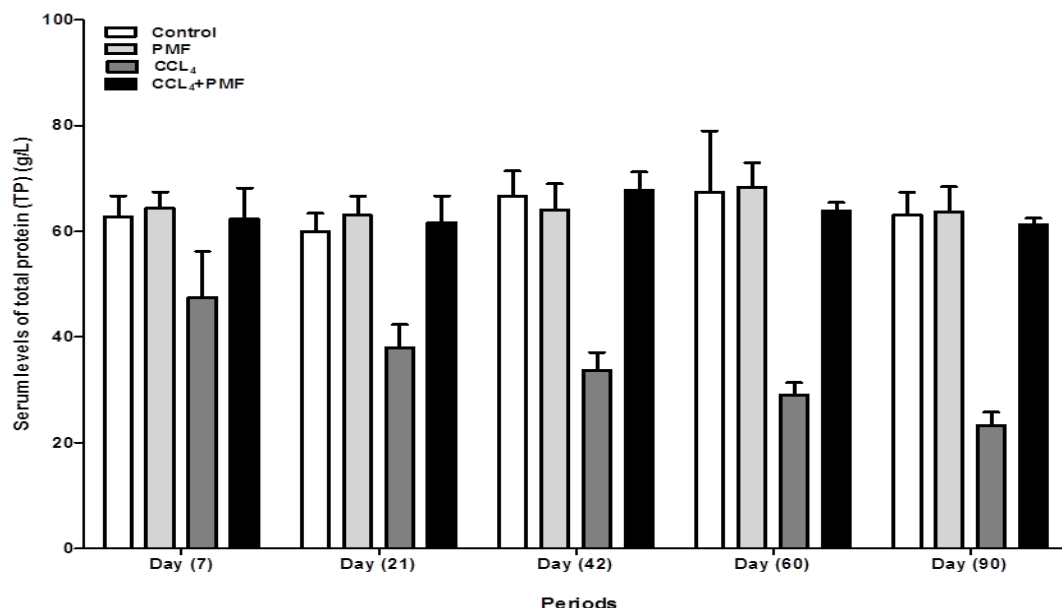


Figure 8: Changes in serum levels of albumin (ALB) (g/L) in different groups at different periods. Data are expressed as mean \pm SD.

3.5. Histological Observation with Hematoxylin and Eosin staining

In this study, the three main areas of the liver structure we investigated (capsule, central vein and portal area) using light microscope we examined the tissue at (7th, 21st, 42nd, 60th and 90th days) here, we only presented the image of 90 days. The image of sections liver of control rats, showed the normal appearance of the liver tissue which is surrounded by thin connective tissue capsules covered by thin endothelial layer of peritoneum. No changes in capsule thickness were observed in photos of samples for all periods (Fig. 10A). Hepatocytes are arranged in plates or cords radiating from the central veins (CV). They have slight acidophilic cytoplasm and rounded vesicular nuclei, some cells are binucleated. The cords are separated by thin walled blood sinusoids lined by endothelial cells, nuclei of Von kupffer cells are occasionally seen (Fig.11 A). At portal areas, the triad; portal vein (PV), hepatic artery (HA) and bile duct (BD) were seen clearly, surrounded by scanty connective tissue with few cells. Hepatocytes near the portal area also showed normal structure (Fig.12A). Compared to control group, the liver cells from rats received oral PMF showed no histological changes in the capsules thickness (Fig. 10B). Blood sinusoids around central veins (CV) appeared normal and non congested. Hepatocytes cords are normal with vesicular nuclei (Fig. 11B). Portal areas showed normal branches of portal vein (PV), hepatic artery (HA) and bile duct (BD). No fibrosis or inflammatory changes were seen at 90 days (Fig. 12B). On other hand the sections of the liver from rats injected with CCL₄ showed many histological changes in structure of the liver tissues. That include marked fibrous thickening of rat liver capsules with deposition of fat or adipose tissue and inflammatory cells, which were the most evident changes at all periods. Sinusoidal congestion was also observed in the region under liver capsules (Fig. 10C). In some sections lipid droplets of varying sizes were observed within hepatocytes especially in those located near the central veins. Lipid deposition was observed at liver samples of 21, 42, 60 and 90 days with duration dependent effect (Fig. 11C1).). Increase in fibrous tissue was observed around the central veins at 21, 42, 60 and 90 days, also most

hepatocytes showed dark cytoplasm and nuclei as an early signs of apoptosis. Von kupffer cell nuclei become prominent (Fig. 11C2). After 90 days a massive focal destruction of liver parenchyma was seen due to hepatocyte degeneration associated with hemorrhage. Nearby cells showed dark small nuclei (Fig.11C2). The most histological changes were observed at portal regions as portal veins (PV) appeared markedly dilated and congested. There was more proliferation of the cells of bile ducts (BD) with peri-duct fibrosis, increased inflammatory and mast cells. Hepatic arteries (HA) showed thickening of their walls and the most evident changes were the increased in apoptotic dark cells located near portal triad (Fig. 12C). The sections of the liver from rats injected with CCL₄ for two days via IP routes then treated with oral PMF after one week, showed that the fibrous thickening that was present in CCL₄ disappeared at 42, 60 and 90 days of treatment (Fig. 10D). Central vein (CV) still congestion at 7 days with presences of apoptotic cells, while after 21, 42, 60 and 90 days liver parenchyma looked normal and there were only some prominences of Von kupffer cells (Fig. 11D). Portal area looked normal compared to control. (Fig. 12D).

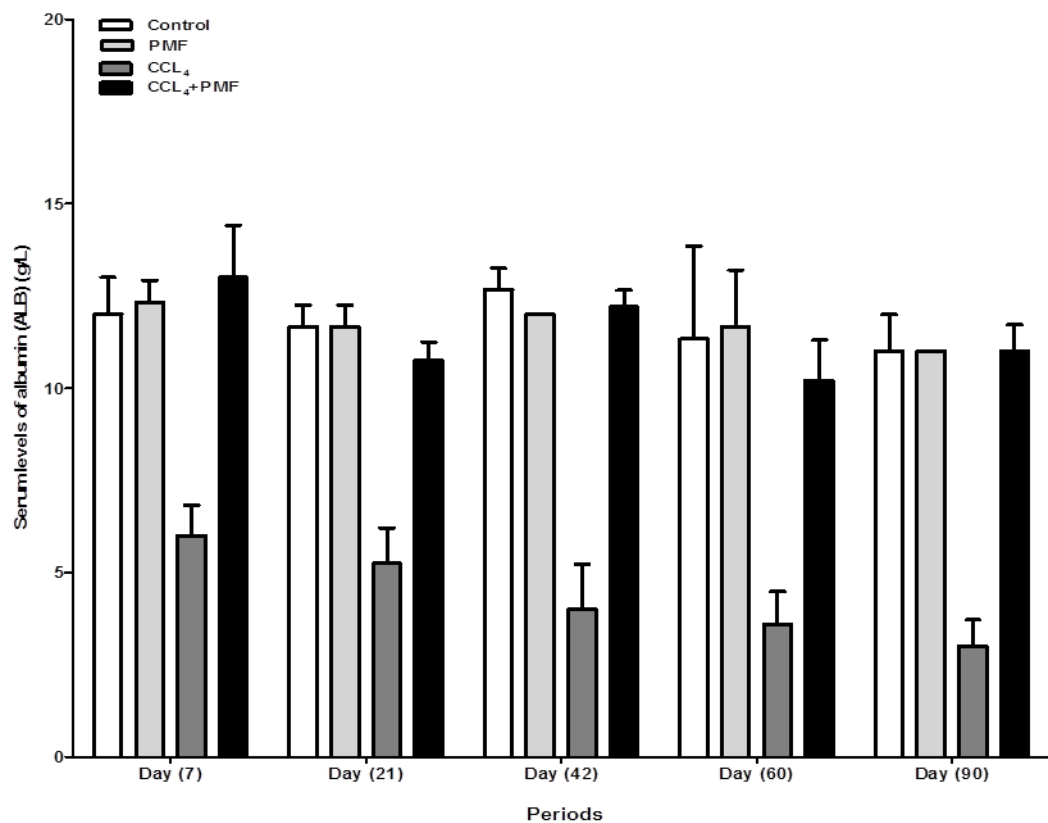


Figure 9: Photomicrograph of liver capsule in experimental groups after 90 days stained by Hematoxylin and Eosin staining (A) Control rat liver (B) Rats treated with oral PMF: showing no changes in capsules thickness (C) Rats injected with CCL₄ showing capsules thickening (double heads arrow) with adipocyte deposition (arrow) and inflammatory cells infiltrate (dotted arrow) (D) Rats administrated with CCL₄ and treated with oral PMF showing that no thickness was observed at the regions of capsule (arrow) Original magnification $\times 400$, bar = 50 μ .

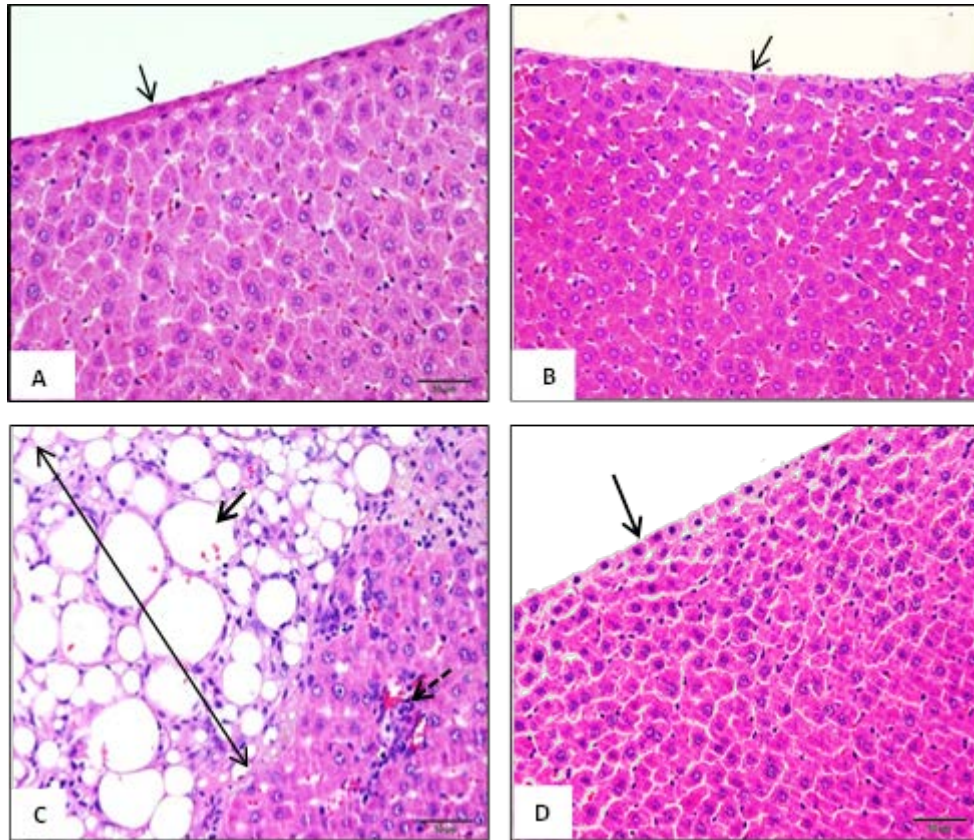


Figure 10: Photomicrograph of liver central vein region in experimental group after 90 days stained by Hematoxylin and Eosin staining (A) Control rat liver showing central veins (CV), hepatocytes (black arrow) extend in the form of plates or cords to the periphery. Cells are separated by thin non congested blood sinusoids which are lined by endothelial cells (dotted arrow). (B) Rats treated with oral PMF showing normal hepatocytes cords with normal vesicular nuclei (black arrow). Central veins (CV) and sinusoids are seen with normal appearance. (C1 and C2) Liver sections of treated rats injected with CCl_4 . (C1) showing lipid deposition of various sizes and density within hepatocytes (arrow) especially near the central veins (CV). (C2) Massive focal destruction of liver parenchyma due to hepatocyte degeneration associated with hemorrhage (star) nearby cells, that showed dark small nuclei (dotted arrow).(D) rats administrated with CCL_4 and then treated with PMF showing that liver parenchyma looked normal and there were only some prominences of Von kupffer cells (dotted arrow). Original magnification $\times 400$, bar = $50\ \mu$.

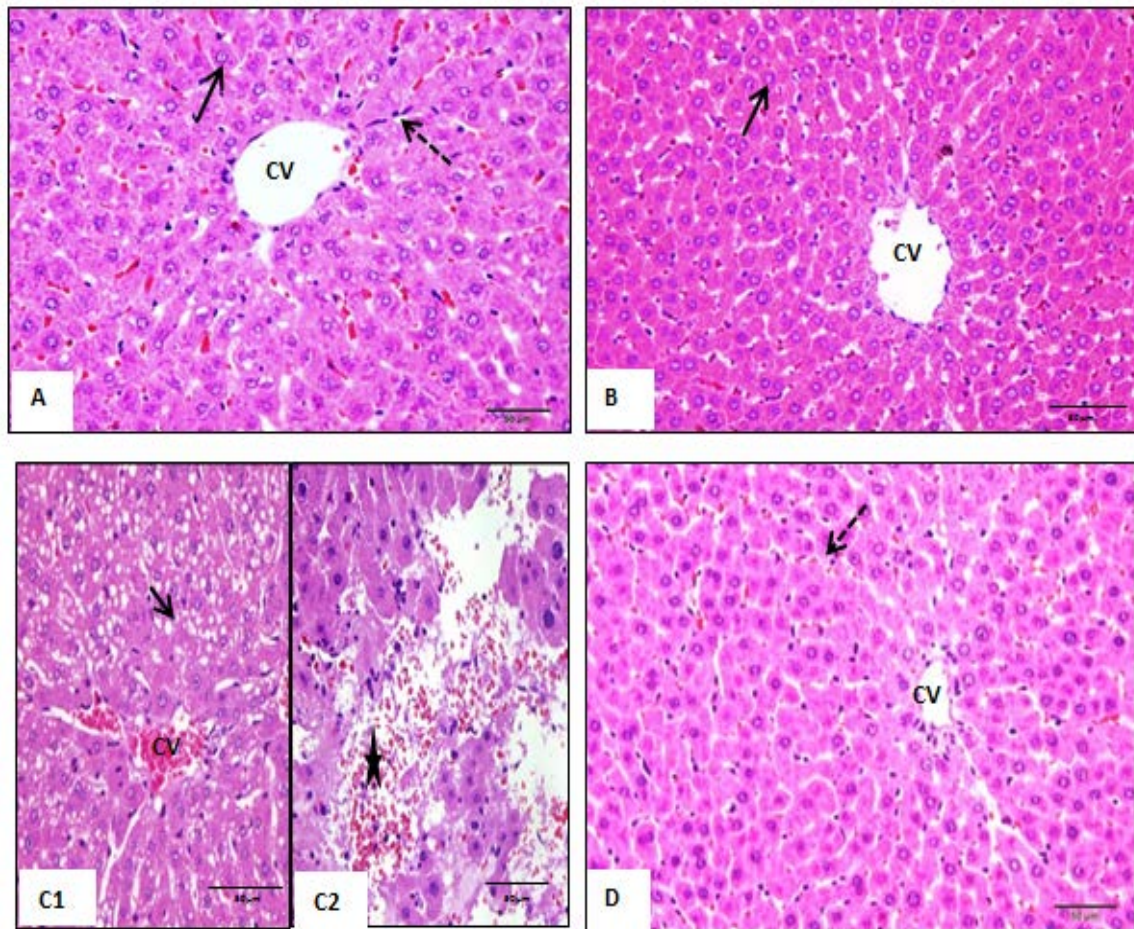


Figure 11: Photomicrograph of liver portal area in experimental group after 90 days stained by Hematoxylin and Eosin staining (A) Control rat liver showing portal vein (PV), hepatic artery (HA) and bile duct (BD). Notice the scanty connective tissue around portal triad. Hepatocytes near the portal area also looked with normal structure (B) portal area of rats treated with oral PMF showing normal portal triad, portal vein (PV), hepatic artery (HA) and bile duct (BD). No fibrosis, bile duct proliferation or inflammatory cells were observed also. (C) In group CCL_4 showing marked changes such as dilatation and congestion of portal veins (PV), increase the number of bile ducts (BD) and increased of fibrous tissues with infiltration inflammatory by mono nuclear and mast cells (dotted arrow). Increased of dark apoptotic cells were also observed (arrow). (D) Liver portal area of rats administrated with CCL_4 treated with oral PMF showing Normal structure compared control group. Original magnification $\times 400$, bar = $50\ \mu$.

3.6. Histological Observation with Masson's trichrome stain

Masson's trichrome stain was used in this study to demonstrate collagen fiber distribution in the same areas (capsule, central vein and portal area) of rats liver. Our results showed that the capsules of liver in control rat are very thin and contain few collagen fibers at all periods (Fig. 13A). Also few fibers were seen around the central veins (Fig. 14A). On the other hand, portal areas showed more deposition of collagen around portal triad (Fig. 15A). No changes observed in collagen content of liver either in the capsules or around the central veins or portal triads in group administrated with PMF compared to control group (Fig. 13,14,15 B). On the other hand,

CCL₄ group showed a thickening of the liver capsules with deposition of adipocytes and mononuclear cells infiltrate in all durations of experiments (Fig. 13C1). Also collagen fiber extension was seen between lobules (bridging) with adipose tissue between liver lobules in some samples (Fig. 13C2). Also an increase in collagen fibers around the central veins was also seen (Fig. 14C). More evident of increase in the collagen fibers were observed around portal triad in CCL₄ group (Fig. 15C). Finally, the collagen fibers around capsule, central vein and portal triads in liver section of rat injected with CCL₄ and then treated with PMF were similar to those of the control group (Fig. 13, 14, 15, D).

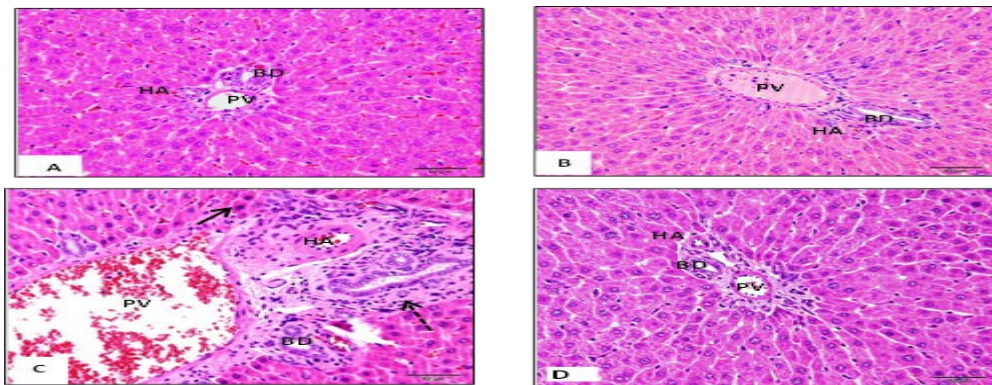


Figure 12: Photomicrograph of liver capsule in experimental group after 90 days stained by Masson's trichrome staining (A) Normal structure of control rats showing few stained collagen fibers at region of capsules (arrow).(B) liver sections of rats treated with oral PMF showing no changes in capsules collagen fibers (arrow).(C1 and C2) Rats injected with CCL₄ for two days at different periods of experiments without receive treatment showing that (C1) there was fibers thickening of the capsules (arrow) with fat cells deposition (dotted arrow).(C2) Deposition of collagen and bridging between liver lobules (arrows).(D) liver sections of rats administrated of CCL₄ and treated with oral PMF showing absence of such thickening in capsules collagen fibers (arrow) Original magnification $\times 200$, bar = 100 μ .

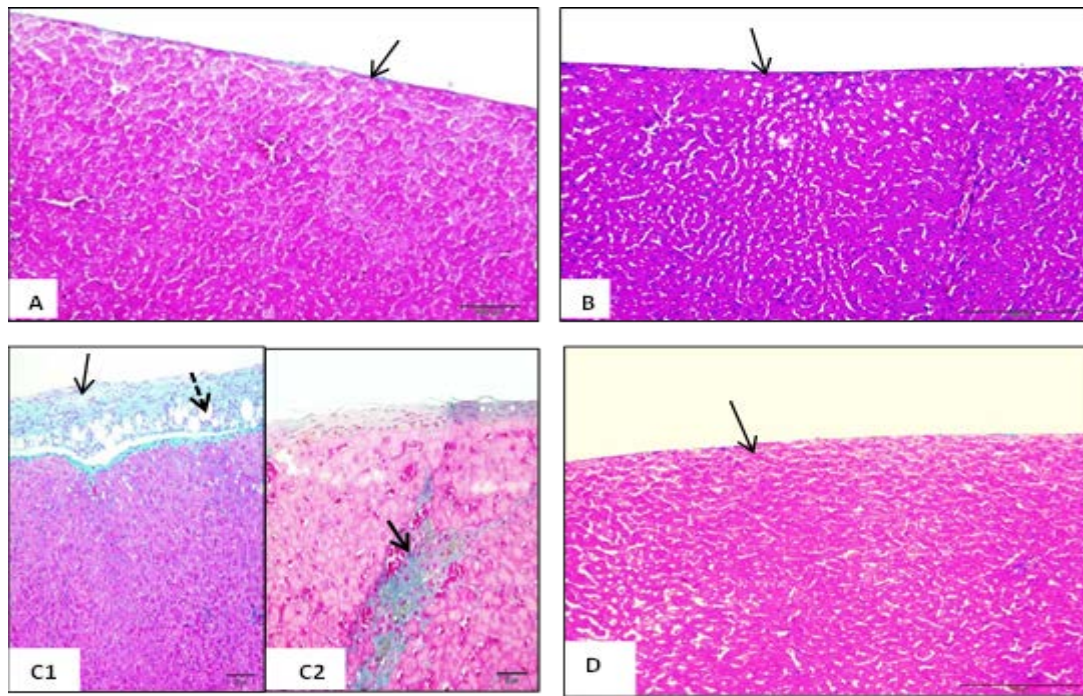


Figure 13: Photomicrograph of liver central vein in experimental group after 90 days stained by Masson's trichrome stain (A) Control rats showing few fibers at region of central veins (CV). (B) Rats treated with oral PMF showing no changes in collagen fibers around central veins.(C) Rats injected with CCl_4 for two days without receive treatment showing clear increased in collagen fibers around the central veins as comparing with control sections.(D) Rats administrated of CCL_4 and treated with oral PMF after showing Normal collagen content around the central veins (CV). Original magnification $\times 200$, bar = $100\ \mu$.

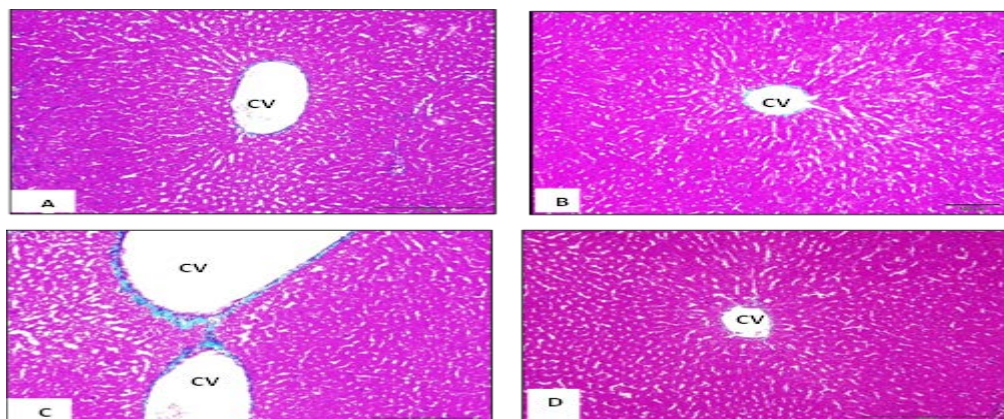


Figure 14: Photomicrograph of liver portal area in experimental group after 90 days stained by Masson's trichrome stain (A) Normal structure of control rat liver (B) Liver portal area of rats treated with oral PMF no apparent changes in collagen content around portal triad (arrow). (C) Rats injected with CCl_4 showing an increase in collagen fibers around portal triad (arrow) at all duration of experiments. Notice the collagen bridging between lobules in some samples (dotted arrow). (D) Rats administrated of CCL_4 treated with oral PMF showing normal collagen compared the control group. Original magnification $\times 200$, bar = $100\ \mu$.

4. Discussion

CCl_4 is one of the most commonly hepatotoxins that has been reported to show many metabolic and morphologic aberrations in the liver of the experimental animals similar to those observed in human viral hepatitis [29,30]. In this study we induced the toxic effect to liver by administration of CCl_4 . In our results, the liver functions tests showed significant decrease in liver enzymes (ALT, AST, ALP and GGT) levels in the group treated with oral PMF. There biochemical results of CCl_4 group showed a damage of liver by an elevation in the levels of serum ALT, AST, ALP and GGT. This indicated an alteration in membrane integrity, hepatonecrosis and as a result releases these enzymes in to the blood circulation similar results were confirmed by [31,32,33,34,35,36,37]. The activities of these enzymes in serum can reflect the degree of liver injury [38,39].

The AST and ALT are the most sensitive indicators of liver injury[40]. ALT enzyme is one of the indices of the degree of cell membrane damage, Whereas AST is one of the indices of mitochondrial damage, since mitochondria contain 80% of this enzyme [41].

Increase in plasma ALP in CCl_4 treated rats could be due to its increased synthesis due to the elevated biliary pressure and subsequent increment in bilirubin [42].

An increase in serum GGT is a defense mechanism reflecting the induction of cellular GGT, when there is oxidative stress [43].

Bilirubin is an endogenous substance and degradation product of hemoglobin was also found to be restored by PMF treated in experimental rats after been significantly elevated in CCl_4 treated rats. Bilirubin is also a measure of hepatotoxicity [44] and could be attributed to impaired hepatic clearance due to hepatic parenchymal damage and biliary obstruction [45] .

This results is supporting the earlier results which showed a similar effect of camel urine and camel milk on hepatotoxicity by CCl_4 [30,46,47,48,49,50,51].

While all liver damaged and then treated animals with oral PMF showed significant increase in total serum protein and albumin to reach the normal levels. However, in CCl_4 group there was decrease in total serum protein and albumin levels. Similar observation were noted in previous studies using camel urine and camel milk on CCl_4 [30,46,47,48,49,50,51,52]. The decrement in total serum protein recorded after CCl_4 treatment was attributed to the decreased number of functional hepatocytes as proved by [33]. In the conclusion, the biochemical results with the noticed increase in the levels of ALT and AST as well as the decrease in the levels of total protein in the serum agreed with all previous studies whose reported that these changes are the major diagnostic symptoms of liver diseases.

Treatment with PMF attenuated the increased level of serum enzymes. This gives a support that PMF are able to restore hepatocytes, accelerate regeneration of parenchyma cells, and protects against membrane fragility and decrease leakage of the enzymes into blood circulation.

In this study, we demonstrated as that PMF restore the normal body weight and liver weight of animal after the major weight loss caused by CCL₄. CCL₄ intoxication has shown a dramatic decrease in of body weight and significant increase in the relative weight of liver than controls. The body weight decrease following CCL₄ injection could be due to either a result of direct toxicity of CCL₄ and / or indirect toxicity related to the liver damage. The metabolic dysfunction due to hepatopathy was believed to be the causative factor for the significant loss in body weight in animals with administration of CCL₄[53]. Additionally, the observed decrease in body weight could be due to the direct effect of CCL₄ on the food intake behavior of the rats based on a study reported that exposure to CCL₄ have significant reduction in their the average feed intake and growth rates [54,55].

Studies that relate to liver weight changes in general are complex. The decrease in liver weight may related to many factors, such as pathogenesis, feeding behavior, fat storage, regulation of energy intake and energy expenditure, as well as hormonal, genetic and psychological influences [56]. When the liver is damaged, it can initiate regenerative actions, thus increasing the weight of liver [57]. If it was heavily damaged, however, liver fibrosis and cirrhosis appear resulting in liver atrophy [58].

The present results showed that oral administration of PMF has positive effect on liver damaged histopathological alterations induced by CCL₄. Such as thickened liver capsules with adipocyte deposition, fibrosis, apoptosis, necrosis, inflammatory cell aggregation and lymphocytes. These histopathological changes are in agreement with previous reports on CCL₄ induced hepatotoxicity [52,59,60,61]. On the other hand these degenerative changes were shown to be minimal or absent with the PMF treatment, periode-dependently. This might be due to re-establishment of the antioxidant defense system in the liver tissue through the antioxidant and hepatoprotective nature of PMF. This indicated the effectiveness of PMF in preventing of CCL₄ toxicity. These observations were in line with previous studies, which investigated the effect of camel urine and camel milk on CCL₄[30,46,47,48,49,50,51,52]. Which reported that the camel urine and camel milk can act as protective agent against liver damage and that could be attributed to the antioxidant activity of camel urine and camel milk on toxicants. Whereas the toxic effect of CCL₄ on hepatocytes is due to its metabolic conversion by the NADPH-cytochrome P450 metabolizing enzyme system to the highly reactive free radical CCl₃. Free radicals cause the peroxidation of polyenoic lipids and the generation of secondary free radicals derived from these lipids [62]. Reactive oxygen species (ROS) affects the antioxidant defense mechanisms, decreases the activity of superoxide dismutase (SOD) that causes liver injury, fibrosis, hepatic necrosis, cirrhosis development and hepatocarcinoma [63]. Thus, the antioxidant activity or the inhibition of the generation of free radicals is important in the protection against CCL₄ which induces hepatopathy [64]. The efficacy of any hepatoprotective drug is dependent on its capacity of reducing the harmful effect or restoring the normal hepatic physiology that has been distributed by a hepatotoxin [30]. In the present work, the histological microscopy examinations of liver tissues revealed that the treatment with PMF suppressed the acute hepatic damage and show improvement of plasmatic and tissue biological parameters for hepatotoxicity.

Therefore, we strongly conclude that PMF have protective effect against hepatotoxicity by inhibiting and reducing

the CCL₄ effect in rats liver. This might be achieved by scavenging or blocking the formation of free radicals generated during CCL₄ metabolism. These positive effects of PMF could be attributed to be bioactive constituents that alleviated the deleterious effect of CCL₄ either by the well-known scavenging action or the antioxidant properties that inhibited lipid peroxidation stabilized the reactive radicals, preserve the cellular integrity and restrain the severity of CCL₄. This effect was also seen in camel urine and camel milk on CCL₄ [30,46,47,48,49,50,51,52].

PMF contains nitrogen, carbon, sulfur, hydrogen, chlorine and bromine, potassium, sodium, Cu, Fe and Zn [65], which are known trace elements essentially required for normal biological functions in human [12]. Most of those elements were proved to have antioxidant effect with protective effect for membranous and cellular integrity may contribute to mitigate the oxidative stress as agreement with [66]. Antioxidants may prevent and/or improve different diseased states [67].

It is worth mentioning, the obtained results showed that the treatment of rat with oral PMF revealed no significant changes in histological compared to control group. This indicated the extent of safety in using PMF in negative control animals, similar observation in cell tissues and healthy volunteers [17,68].

The market chemically structures medications have many harmful side effects for example Ribavirin and interferon. Ribavirin is given in conjunction with interferon for the treatment of hepatitis C. The most common adverse effects of interferon and ribavirin are hematologic, dermatologic, neurologic, immunologic, gastrointestinal, pulmonary, cardiovascular, and ocular. The common side effects associated with interferon-ribavirin combination therapy, including myalgias, fever, depression and cytopenia. There are less common side effects that can cause significant morbidity and mortality if they are not recognized expeditiously [69].

A case of interstitial granulomatous pneumonitis that occurred after administration of interferon and ribavirin therapy, Proposed mechanisms for the activity and pulmonary toxicity associated with interferon include inhibition of suppressor T cells, enhancement of cytotoxic T cells, induction of proinflammatory cytokines, and exaggerated release of fibrinogenic cytokines, such as platelet-derived growth factor and transforming growth factor- β , leading to lung tissue fibrosis. Also proposed that IFNs can contribute to exacerbations of asthma through the activation of The lymphocytes, which results in increased production of IFN- γ and cytokines [70]. Chronic cough associated with interferon/ribavirin therapy for hepatitis C. Previous studies have observed that cough occurs more commonly in patients receiving the combination of interferon and ribavirin [71].

The nephrotoxicity associated with interferon therapy for chronic hepatitis C infection has not been clearly defined. He described a patient with chronic hepatitis C infection who developed the nephrotic syndrome during treatment with interferon alfa and ribavirin [72]. Renal biopsy revealed focal segmental glomerulosclerosis. Interferon therapy for hepatitis C can result in renal complications as interstitial nephritis, minimal change disease and immune complex glomerulonephritis [73].

The neurotoxicity of commonly used hepatic drugs. Severe neurological effects related to these therapies have been reported [74]. Also interferons, ribavirin are therapeutic options commonly encountered in the treatment of

hepatitis as indicated by many studies indicated that Interferon and ribavirin combination therapy for chronic hepatitis C produces hemolytic anemia [75,76,77].

The effectiveness of treatments such as interferon and ribavirin are inconsistent at best and the incidence of side effects is profound. All too often the treatment is worse than the disease [78,79]. Physicians and patients are thus in need of effective therapeutic agents with a low incidence of side effects [80]. In contrast with these available medications in the market, PMF is considered as antihepatitis drug with no significant side effects. This agrees with previous studies on PMF by [17,18,19].

5. Conclusions

In conclusion, the results of the present study indicated that under the present experimental conditions, PMF provide a protective effect against liver damage in rats and improve the physiological and the histopathological alterations caused by CCL₄. This study suggest that the effects of this substanc against CCL₄ induced hepatitis, possibly due to antioxidant properties of their natural chemical constituents.

6. Recommendations

This study is the first investigations that apply scientific methology to look at how the PMF its role in the protection action against physiological disturbances and histopathological alterations in hepatitis induced by CCL₄. Additional physiological and histopathological investigations are needed to explore the possible use of different doses of these substances as potential natural therapeutic agents in therapy of hepatitis, liver fibrosis against CCL₄ and other chemical agents or fibrogenic factors.

As a future work it will be more applicable if using viral hepatitis models in animals and treat these animals with PMF.

Acknowledgment

I would like to express my appreciation to my supervisor Prof. Faten Khorshid, Professor of Cell Engineering and Cell Biology, Faculty of Science, Biology Department, King Abdulaziz University, Co-Advisor Prof. Soad Shaker, Professor of Cytology and histology, Faculty of medicine, Anatomy Department, King Abdulaziz University and Prof. Samah Noor, Professor in Molecular and Medical Microbiology, Faculty of Science, Biology Department, King Abdulaziz University for helpful suggestions during this project.

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