EFFECT OF EARLY AND LATE PLATELET RICH PLASMA THERAPY IN EXPERIMENTAL CHRONIC LIVER DISEASE: ROLE OF LIPID PROFILE.

Rania Salah Mansour, Gehane M. Hamed, Aliaa Abdelmoneim and Soha M. Yousry

Physiology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt

Corresponding author: Rania Salah Mansour

Mobile: +20 01006422195
e.mail: *dr_raniasalah@yahoo.com*

Received: 29/1/2023
Accepted: 2/2/2023

Online ISSN: 2735-3540

ABSTRACT:

Background: *Chronic liver disease is a public health problem in Egypt. Thioacetamide (TAA), a hepatotoxin causing damage resembling that of the human, allows proper evaluation of new therapeutic approaches such as platelet rich plasma (PRP). Lipid profile was suggested as a prognostic indicator in patients with advanced liver disease.*

Aim of work: *To evaluate the effect of early and late PRP treatment on the markers of liver injury including lipid profile, in Thioacetamide-induced chronic liver diseased rats.*

Materials and methods: *Sixty female albino rats were divided into* ***4 weeks group and 6 weeks group;*** *each group was further divided into 3 subgroups;*

TAA group: *given TAA twice weekly for 4 weeks in both the 4 and 6 weeks groups.*

TAA-PRP group: *PRP was given, immediately after TAA for 4 weeks in the 4 weeks early treated group or started 2 weeks after TAA for 4 weeks in the 6 weeks late treated group.*

Control group*.*

Biochemical assessment included ALT, AST, Albumin, lipid profile, Malondialdehyde (MDA), Total antioxidant capacity (TAC), and hepatic MDA and glutathione peroxidase (GPx).

Results: *Both TAA groups showed significant elevation of serum ALT, AST, serum and hepatic MDA and significant reduction of serum albumin and lipid profile. Early and late treatment with PRP, significantly improved the liver enzymes and serum and hepatic MDA. Albumin was normalized in early treated group while plasma TC and LDL-C were normalized in late treated group.*

Conclusion*: PRP treatment successfully improved liver enzymes, lipid profile, albumin as well as oxidative stress.*

Keywords: *chronic liver disease, thioacetamide, lipid profile, platelet rich plasma.*

INTRODUCTION:

Liver fibrosis is a public health problem with high morbidity and mortality. It occurs in most types of chronic liver diseases [1]. Liver fibrosis usually advances into liver cirrhosis and liver cell failure [2]. "Chronic liver disease" includes many conditions with different etiologies[3] which threatens the wellness of billions of people [4].

The liver plays an important role in lipid metabolism including all stages of lipid synthesis and transportation. Therefore, abnormal lipid profile is expected in patients with severe liver dysfunction [5]. Many studies suggested the application of lipid profile as a prognostic indicator in patients with advanced liver disease[6, 7, 8, 9].

Thioacetamide (C2H5NS; TAA) an organosulfur compound was reported to induce rat liver cirrhosis which is very similar to the human disease and histologically similar to that caused by viral hepatitis infection [11]. It provides an excellent animal model for liver fibrosis and cirrhosis [10].

Some studies have reported that platelets can promote liver regeneration [12]. However, till the beginning of the 21st century, effect of platelets on liver regeneration was not fully studied. **Shoeib et al., (2018)[13]** found that platelet rich plasma (PRP) could protect against TAA-induced liver damage and suggested that this effect may be mediated by improving oxidative stress, suppressing the inflammatory and fibrotic response and preserving the liver histopathological architecture. Moreover, **Salem et al., (2018)[14]** considered that PRP could be a promising therapeutic approach for liver regeneration and prevention of fibrosis where PRP markedly improved the changes in liver enzymes, increased the anti-apoptotic markers and significantly down-regulated the fibrosis-related genes.

AIM OF WORK:

To evaluate the effect of early and late PRP treatment on the markers of liver injury including lipid profile, in Thioacetamide-induced chronic liver diseased rats.

MATERIAL AND METHODS:

Experimental animals:

This study was performed on 60 Albino female rats purchased from the Research Institute of Ophthalmology (Giza) and were maintained in the *Physiology Department Animal House* under standard conditions of boarding and feeding. The given diet consisted of bread, milk and green vegetables with free access to water.

Experimental protocol:

This study was approved by the ethical committee of Faculty of Medicine, Ain Shams University.

Rats included in the present study were allocated into the following groups:

A) Four-weeks groups:

A1. Control group: (n=10): Rats of this group received intraperitoneal injection (IP) normal saline twice weekly and were studied by the end of the 4th week.

A2. Thioacetamide group (TAA-4w group): (n=10): Rats of this group received IP injection of TAA (200 mg/kg) twice weekly for 4 weeks for induction of liver cirrhosis (chronic liver damage) [15].

A3. Thioacetamide - platelet rich plasma early treated group (TAA-PRP early treated group): (n=10):

To clarify the possible protective effects of platelet rich plasma (PRP), rats of this group were given IP of TAA (200 mg/kg) twice weekly followed by immediate administration of PRP twice weekly for 4 weeks and rats were studied by the end of the 4thweek.

B) Six-weeks groups:

B1. Control group (n=8): Rats of this group received IP injection of normal saline twice weekly for 6 weeks.

B2. Thioacetamide group (TAA-6w group): (n=11): Rats of this group were subjected to IP injection of TAA (200mg/kg) twice weekly for 4weeks [15] and were studied by the end of 6th week.

B3. Thioacetamide - platelet rich plasma late treated group (TAA-PRP late group) (n=11): To clarify the possible therapeutic effects of platelet rich plasma (PRP), rats of this group were given TAA twice weekly for 4 weeks, then, starting 2

weeks after TAA, PRP was given twice weekly for 4weeks and rats were studied by the end of the 6th week.

Experimental chronic liver disease: For induction of experimental chronic liver disease, Thioacetamide **(C2H5NS; TAA)** (Sigma, USA) given by intraperitoneal (i.p.) injection at a dose of 200 mg/kg twice weekly for 4 weeks in both 4 and 6 weeks groups. [15]

Preparation of Platelet Rich Plasma (PRP):

25 rats were purchased and maintained in the animal house to be used for preparation of Platelet rich plasma (PRP), approximately 5ml of blood was used for preparation of 1ml PRP.

-PRP was freshly prepared prior to injection using steps according to ***(Hamilton et al., 2012)[16].***

-Ether was used to anaesthetize rats, blood was collected through aortic cannulation in a tube containing 3.8% sodium citrate (9 parts of blood to 1 part of sodium citrate).

-Then, the blood was centrifuged at 1000 rpm for 5 min for obtaining PRP which was separated in another tube.

-A second centrifugation of PRP at 3000 rpm for 15 min to obtain the platelet pellet (concentrated platelets).

-Excess plasma above the pellet was drawn off & platelets were gently resuspended in 1 ml residual volume of plasma.

-CaCl2 (25 mM) was added in a ratio 1/10 (1part CaCl2 to 9 parts plasma), then incubated in a water bath (37˚) for an hour to facilitate growth factors release.

-Finally, centrifugation at 4000 rpm for 10 min to precipitate the platelet membrane fragments and the supernatant was pipetted, ready for injection.

-Each rat was injected subcutaneously in a dose of 0.5 ml /kg twice weekly for 4 weeks.

Experimental studies: Rats in all studied groups were subjected to:

-Measuring body weight (initial & final)

-Measuring serum levels of

- Albumin, which was determined by a colorimetric BCG method according to ***Doumas et al. (1971)[17]*** and ***Doumas and Biggs (1972)[18],*** using kits supplied by ***Greiner Diagnostic Gmbh***, **Germany.**
- Alanine transferase (ALT), This was determined by an optimized Kinetic method according to ***Bergmeyer et al (1986)[19]***, using kits supplied by ***Greiner Diagnostic Gmbh***, **Germany**.
- Aspartate transferase (AST), which was determined by an optimized Kinetic method according to ***Bergmeyer et al (1986)[19]***, using kits supplied by ***Greiner Diagnostic Gmbh***, **Germany**.
- Malondialdehyde (MDA), which was measured in serum by colorimetric method according to the method described by ***Satoh (1978)[20],*** and ***Ohkawa et al. (1979)[21],*** using kits supplied by **Bio-diagnostic, Egypt**.

-Measuring plasma levels of lipid profile including

- Triglyceride level, which was determined by an enzymatic colorimetric method according to ***Rifai et al. (1999)***[22], using kits (GPO-PAP) supplied by ***Greiner Diagnostic Gmbh,*** **Germany.**
- Total cholesterol, which was determined by an enzymatic colorimetric method according to ***Rifai et al. (1999)[22]***, using kits (CHOD-PAP) supplied by ***Greiner Diagnostic Gmbh,*** **Germany.**
- High density lipoprotein cholesterol was determined by an enzymatic colorimetric method according to the method described by ***Rifai et al. (1999)[22]***, using kits supplied by ***Greiner Diagnostic Gmbh***, **Germany**.
- Calculation of low-density lipoprotein according to the following equation: LDL-C = (TC) – (HDL-C) – (TG/5) ***[23].***

- Total antioxidant capacity (TAC) which was carried out by an enzymatic colorimetric method[24], using kits supplied by **Bio-diagnostic, Egypt**.

-Measuring Hepatic levels of

- GPX Glutathione peroxidase by a UV method described by **Paglia and Valentine (1967)[25],** using kits supplied by **Bio-diagnostic, Egypt**.
- Malondialdehyde (MDA) measured in liver tissue homogenate as described before.

Histopathological Analysis of liver cells

Light microscopic study:

The excised 2-3 mm thick slices of the left lobe of the liver were fixed in 10% formalin solution immediately after removal. The specimens were dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin. Serial sections of 5 um thick were cut and stained with Hematoxylin and Eosin[26].

Statistical Analysis:

Data are presented as the mean ± standard error of mean (SEM). The significance of differences was determined by one-way ANOVA and post hoc test for multiple comparisons using SPSS 20.0 software. Correlation coefficients were calculated by linear regression analysis using the Least Square Method. A p-value of $P < 0.05$ was considered statistically significance.

RESULTS:

Table (1) Showing the **final body weight** (bw) and its **% of change** from initial in all studied groups. The 4wks TAA and early PRP treated group had significantly lower mean bw and its % of change (decrease) compared to their controls, the % of decrease in bw in the treated group was significantly lower than the untreated group. While, in the 6 wks group, both TAA and late PRP treated rats did not show any significant difference in their final bws when compared to the control rats. However, the percent change in bws in TAA showed significant change, compared to the control rats. As well, the percent change in bws of PRP rats showed significant change when compared to TAA rats.

Group	*Initial* weight (g)	*Final* weight (g)	*% Change*
Control-4w Mean ±SEM	240.50 ±4.44	255.50 ±4.44	6.30 ±0.97
TAA-4w Mean ±SEM	252.00 ±7.12	217.50[a] ±7.86	-13.68[a] ±2.01
PRP-early ttt Mean ±SEM	241.00 ± 6.94	230.00[a] ±7.26	-4.17 [ab] ±2.78
Control-6w Mean ±SEM	204.38 ±8.15	218.75 ±10.08	6.88 ±1.33
TAA-6w Mean ±SEM	209.55 ±11.09	199.09 ±8.86	- 4.37[a] ±1.98
PRP-late ttt Mean ±SEM	205.45 ± 9.83	210 ±7.63	2.83[b] ± 1.99

a: Significance of difference from their respective control rats, calculated by LSD at P < 0.05 for unpaired data.

b: Significance of difference from their respective TAA rats calculated by LSD at P < 0.05 for unpaired data.

Table (2) Showing the mean values of the **liver enzymes**, **alanine transaminase and aspartate transaminase (ALT and AST)** and **albumin** in all studied groups. ALT and AST were significantly elevated among the 4 weeks TAA rats compared to the control rats, however the early PRP treated rats liver enzymes levels were insignificantly

changed compared to the controls and significantly decreased when compared to TAA rats. On the other hand, the serum albumin levels were significantly decreased in both the TAA rats and the early treated PRP rats when compared to the control rats.

The 6 wk group showed that the liver enzymes (ALT and AST) were significantly elevated in the TAA rats compared to the control rats, however the PRP rats liver enzymes levels were non significantly changed compared to the controls and significantly decreased when compared to TAA rats. On the other hand, the serum albumin levels were significantly decreased in both the TAA rats and the treated PRP rats when compared to the control rats.

In comparison to the TAA of 4 wk group, the TAA of 6 wk exhibited less elevation in liver enzymes (AST and ALT), whilst Albumin level showed more elevation in 4 wk treated PRP rats when compared to their respective 6 wk rats.

Liver functions tests	4 weeks			6 weeks		
	Control	TAA	PRP Early ttt	Control	TAA	PRP Late ttt
Albumin (g/dl)	3.70 ±0.31	2.69[a] ±0.38	3.66[b] ±0.25	4.04 ±0.30	2.51[a] ±0.23	2.62[ac] ±0.17
AST (U/L)	31.89 ±3.07	70.08[a] ±5.34	42.68[b] ±4.88	23.56 ±3.35	42.97[ac] ±2.91	30.70[b] ±5.89
ALT (U/L)	16.59 ±1.10	39.11[a] ±4.71	16.75[b] ±1.81	13.48 ±0.76	29.19[ac] ±1.39	12.26[b] ±0.97

a: Significance of difference from their respective control rats, calculated by LSD at P < 0.05 for unpaired data.

b: Significance of difference from their respective TAA rats calculated by LSD at P < 0.05 for unpaired data.

c: Significance from their respective 4 weeks rats, calculated by LSD at P < 0.05 for unpaired data.

Concerning **lipid profile, table (3)** showed that the lipid profile in both the 4 wks TAA and PRP groups showed a significant depression in their plasma **triglycerides (TGs)** , **total cholesterol (TC)** and **high density lipoprotein cholesterol (HDL-C)** when compared to the control group, while the **low density lipoprotein cholesterol (LDL-C)** values were insignificant. Moreover, all the parameters were insignificantly different between the TAA and PRP early treated group.

The 6 wks group showed that plasma triglycerides levels were significantly decreased in both TAA and PRP late treated rats compared to their controls. TC was also significantly decreased in the TAA rats. However, the treated PRP rats TC level showed insignificant change compared to controls while, it was significantly higher when compared to the untreated TAA rats. On the other hand, both plasma levels of HDL and LDL cholesterol in TAA rats did not show significant change from their controls. However, plasma LDL cholesterol level showed significant increase in the PRP rats compared to the TAA rats.

Moreover, TAA of 6 wks rats showed more decline in plasma LDL-C level when compared to their respective 4 wks rats. While PRP of 6 wks rats showed more increase in plasma HDL-C level when compared to their respective 4 wks rats.

	4 weeks			*6 weeks*		
	Control	***TAA***	***PRP early ttt***	***Control***	***TAA***	***PRP late ttt***
TGs (mg/dl)	61.09 ±10.18	27.51[a] ±7.71	36.43 [a] ±7.21	87.71 ±11.82	34.61 [a] ±3.99	33.74 [a] ±2.71
TC (mg/dl)	121.82 ±7.27	88.79[a] ±4.77	94.19 [a] ±14.10	103.75 ±5.63	77.29 [a] ±4.52	111.53[b] ±8.77
HDL-C (mg/dl)	57.61 ±4.72	34.58[a] ±3.88	35.09 [a] ±5.50	55.75 ±3.80	46.42 ±5.70	52.68[c] ±5.49
LDL-C (mg/dl)	50.65 ±5.62	49.09 ±3.73	50.91 ±12.59	35.12 ±8.33	25.82 [c] ±5.25	54.84[b] ±5.43

a: Significance of difference from their respective control rats, calculated by LSD at P < 0.05 for unpaired data.

b: Significance of difference from their respective TAA rats calculated by LSD at P < 0.05 for unpaired data.

c: Significance from their respective 4 weeks rats, calculated by LSD at P < 0.05 for unpaired data.

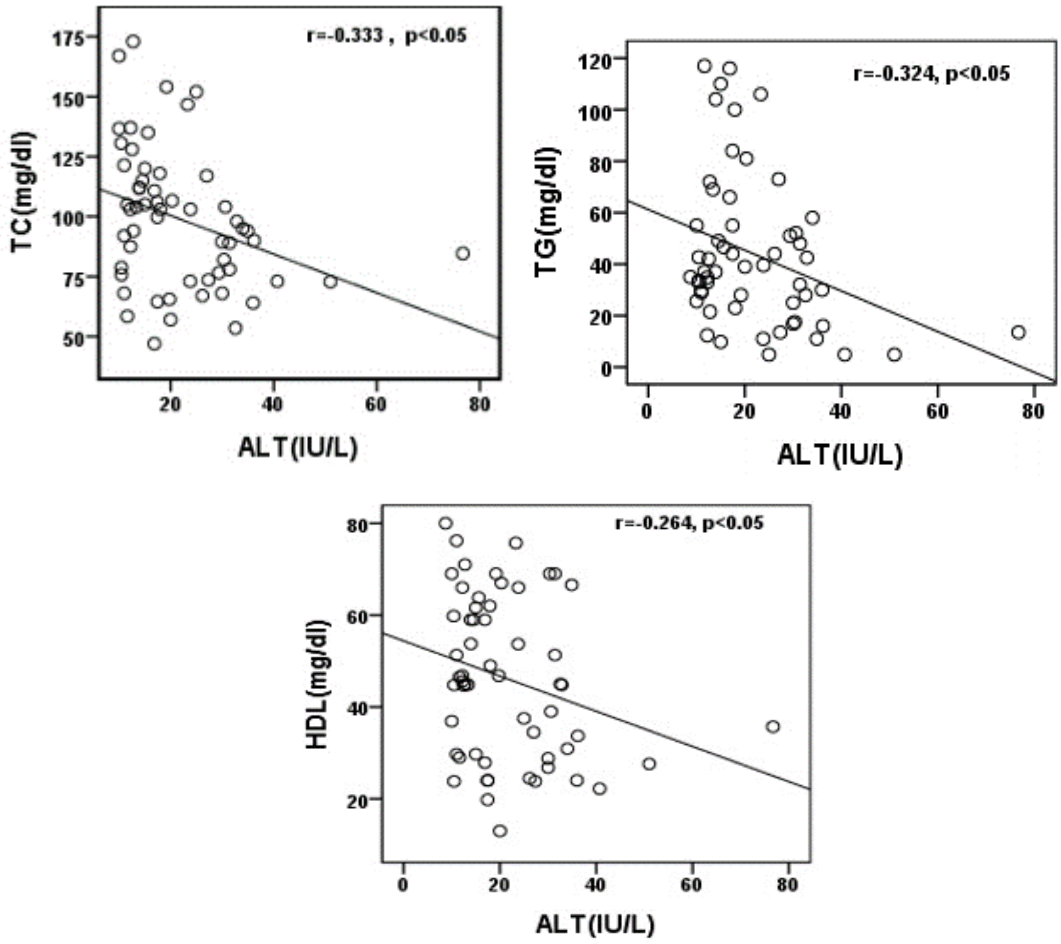
Table (4) showed the **oxidative markers** changes in all studied groups. There were significant elevations in both **serum and hepatic malondialdehyde (MDA)** levels in both 4 and 6 wks TAA rats when compared to their respective control rats. After treatment with the PRP whether early or late treatment, both parameters became significantly decreased compared to TAA rats and insignificant from controls. plasma **total antioxidant capacity** (TAC) showed insignificant differences among the 4 wks group while it was significantly lower in the 6 wks TAA rats compared to control rats, however the changes in the late treated rats with PRP were statistically insignificant. On the other hand, **hepatic glutathione perioxidase (GPX)** levels were insignificantly different among the 6 studied groups while Plasma TAC level of TAA 6 wks rats showed significant decrease from its respective 4 wks rats.

	4 weeks			**6 weeks**		
	Control	**TAA**	**PRP early ttt**	**Control**	**TAA**	**PRP late ttt**
TAC (mM /L)	6.85 ±0.14	6.73 ±0.19	6.42 ±0.20	5.89 ±0.73	4.44 [ac] ±0.38	5.62 ±0.70
Serum MDA (nmol/ml)	1.16 ±0.16	4.25 [a] ±0.82	2.17 [b] ±0.63	1.54 ±0.35	4.20 [a] ±0.51	1.63 [b] ±0.52
Hepatic MDA (nmol/g)	100.94 ±5.94	157.76 [a] ±8.68	111.69 [b] ±11.30	102.45 ±10.34	177.57 [a] ±10.69	98.63 [b] ±6.72
Hepatic GPX (U/g)	491.19 ±69.80	509.62 ±73.01	494.28 ±64.92	502.88 ±114.45	509.55 ±34.63	576.92 ±55.97

a: Significance of difference from their respective control rats, calculated by LSD at P < 0.05 for unpaired data.

b: Significance of difference from their respective TAA rats calculated by LSD at P < 0.05 for unpaired data.

c: Significance from their respective 4 weeks rats, calculated by LSD at P < 0.05 for unpaired data.



(Figure 1): Correlations between serum levels of ALT with plasma TC, TGs and HDL in the studied groups: demonstrating significant negative correlation with each of the mentioned parameters.

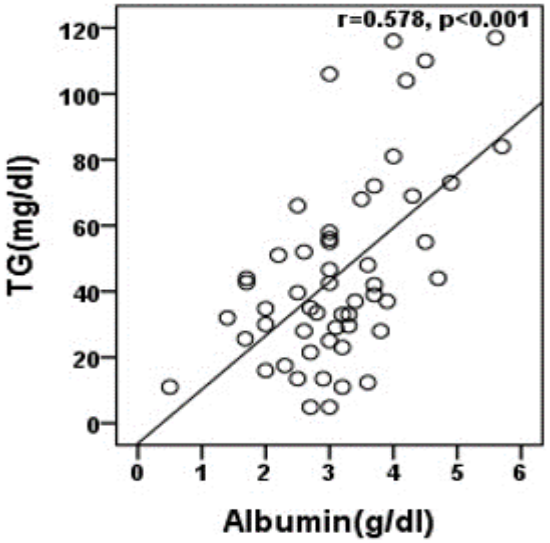


Figure (2) Correlations between serum levels of Albumin with plasma TGs in the studied groups: demonstrating significant positive correlation.

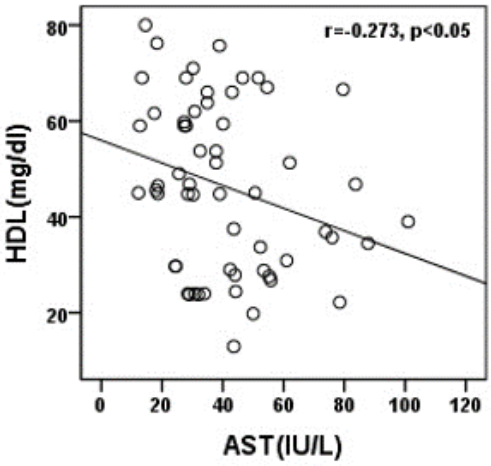


Figure (3) Correlations between serum levels of AST with plasma levels of HDL in the studied groups: demonstrating significant negative correlation.

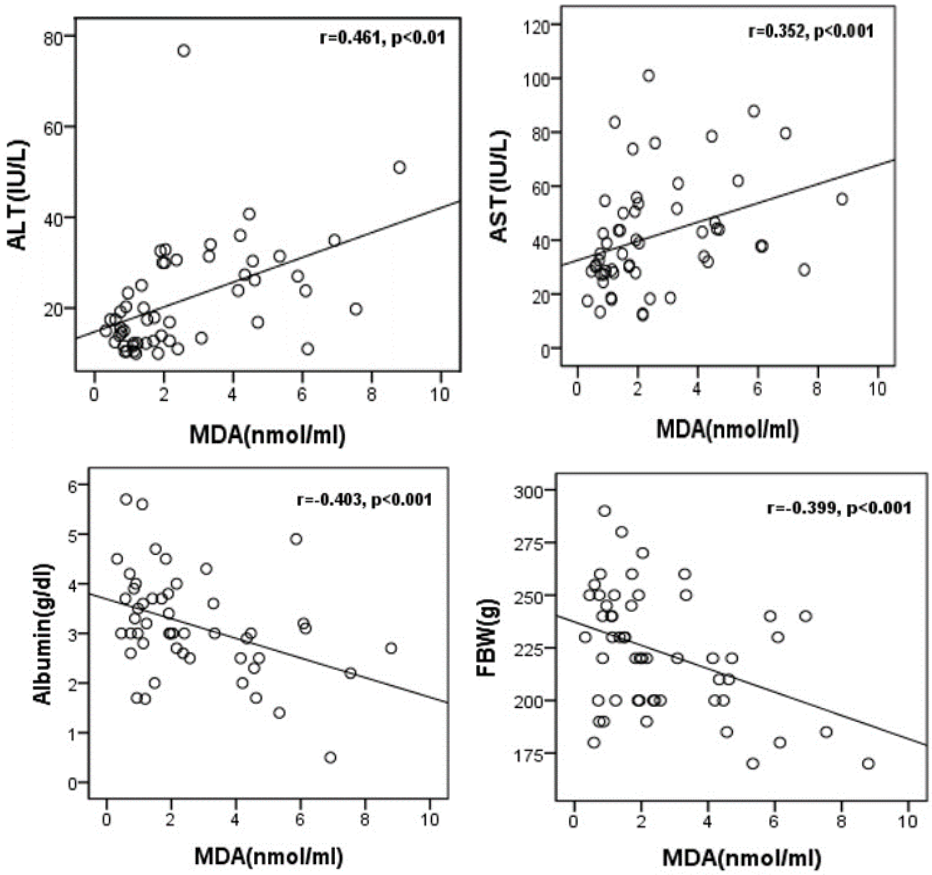


Figure (4) Correlations between serum levels of MDA with serum ALT, AST, Albumin and final body weight (FBW) in the studied groups: demonstrating significant positive correlation with both ALT and AST, and significant negative correlation with each of the Albumin and final body weight (FBW).

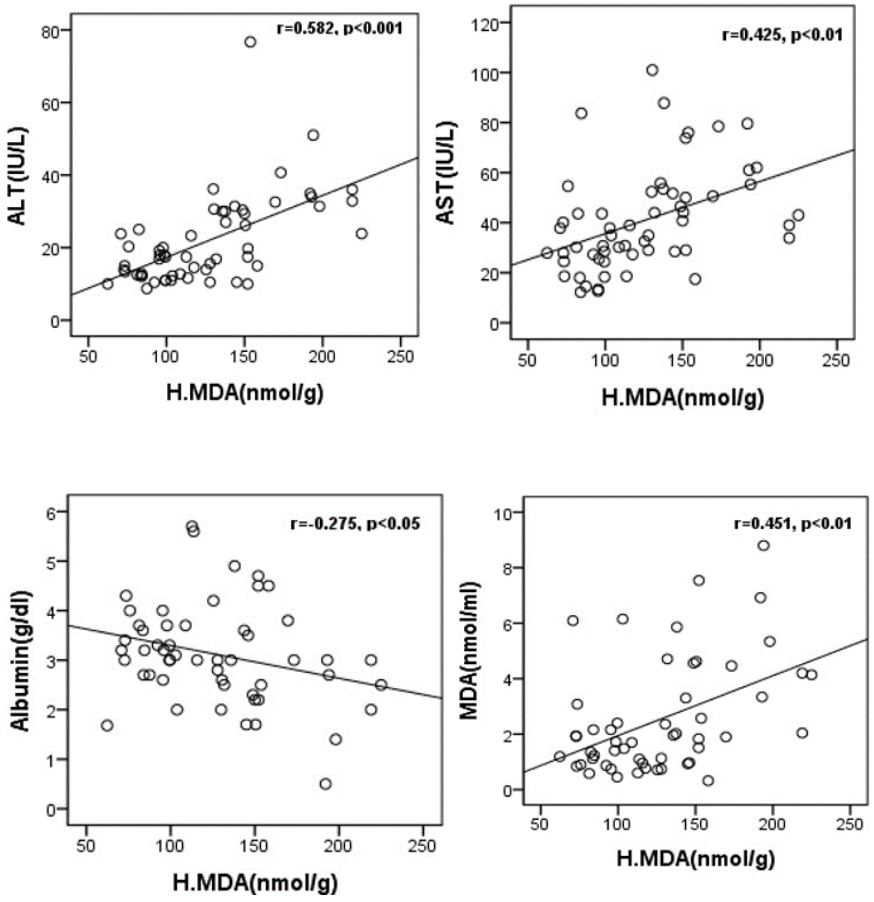


Figure (5) Correlations between hepatic levels of MDA with serum ALT, AST, Albumin and MDA in the studied group: demonstrating significant positive correlation with each of serum levels of ALT, AST and MDA. However, significant negative correlation was demonstrated with Albumin.

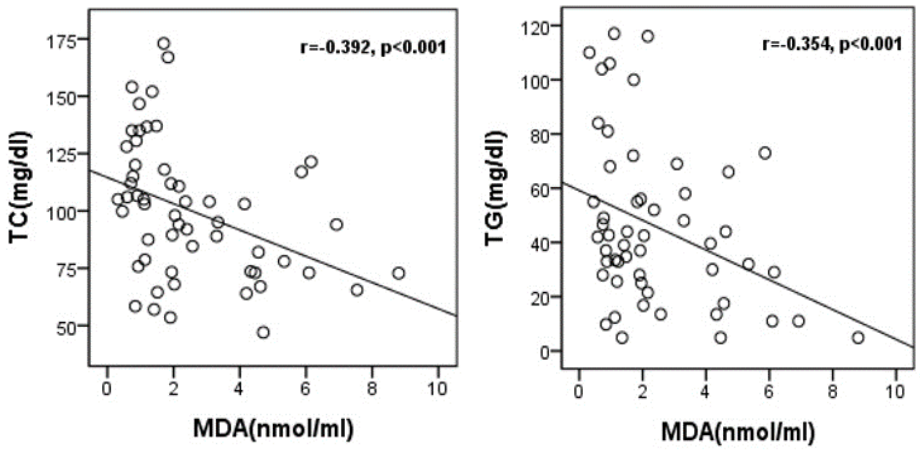


Figure (6) Correlations between serum levels of MDA with plasma levels TC, TGs in the studied groups: demonstrating significant negative correlation with both TC and TGs.

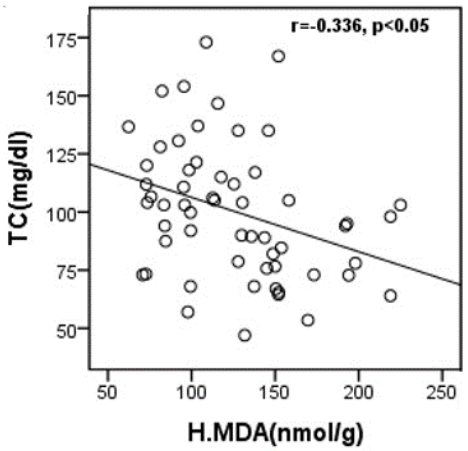


Figure (7) Correlations between hepatic levels of MDA with plasma levels of total Cholesterol (TC) in the studied groups: demonstrating significant negative correlation.

Histopathological studies:

Figure (8): Photomicrographs of liver sections:

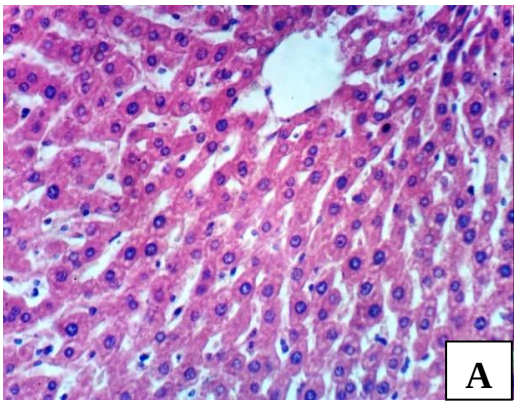
(A) Control group: Showing the central vein surrounded by branched cords of hepatocytes. Cells appear polygonal in shape having eosinophilic cytoplasm with central, rounded and vesicular nuclei. In-between cords, blood sinusoids are lined by flat endothelial cells. (H & E X 400).

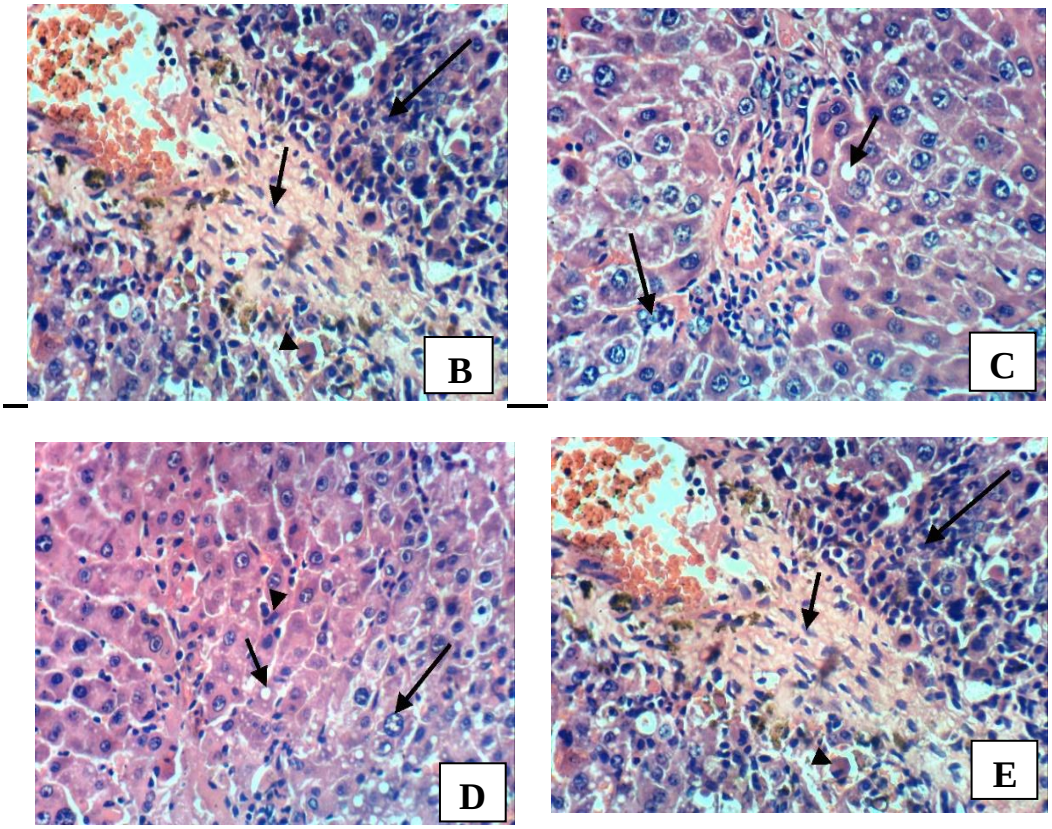
(B) 4 wks TAA group: Showing fibrosis in the portal triad (short arrow), portal infiltration with mononuclear cells (Long arrow) and apoptosis of hepatocytes. Also, large swollen and vacuolated hepatocytes with eccentric nuclei (arrowhead). (H & E X 400).

(C) 4 wks PRP -treated group: Showing less vacuolated hepatocytes with moderate eosinophility near to the portal area (short arrow) with decrease in the inflammatory cell infiltration compared to TAA group. Also, fatty change of hepatocytes and oval cells proliferation (long arrow), (H & E X 400).

(D) 6 wks TAA group: Showing focal necrosis of hepatocytes associated with mononuclear cells infiltration (short arrow), focal haemorrhage and haemosidrosis (long arrow), (H & E X 400).

(E) 6 wks PRP treated group: Showing less vacuolated and less eosinophilic hepatocytes between the central vein and the portal area (short arrow) nearly comparable to control group, with minimal inflammatory cell infiltration compared to TAA group. Also, showing karyomegally (long arrow) and oval cells proliferation (arrowhead), (H & E X 400).





DISCUSSION:

The rise in ALT, and AST after TAA administration might be explained by necrosis or membrane damage which lead to leak of these enzymes into the circulation [27, 28; 29]. It is well-known that they are serum markers of liver injury, where AST indicates chronic liver injury while ALT indicates acute injury) [30]. Treatment with PRP significantly lowered the ALT and AST activities in PRP rats of both 4 weeks and 6 weeks groups reflecting the decline in the damaging effects of hepatotoxicant. These findings agreed with ***Salem et al. (2018)***[14] who concluded the hepatoprotective potential role of PRP. The damaging effects of TAA on hepatocytes and protective effect of PRP were evident in our histopathologic results.

In the present study, the untreated TAA rats of both 4 weeks and 6 weeks groups exhibited significant decreases in serum albumin concentrations when compared to their respective controls. These results were in agreement with ***Rao et al. (2014)***[31] who reported a decrease in serum albumin in TAA-injected rats, which reflected the severity of liver toxicity. The decrease of serum albumin may be caused by increased degradation of abiquitin-associated protein induced by TAA toxic stress [32].

Treatment of TAA intoxicated rats of the 4 weeks group with PRP resulted in normalization of serum albumin. A similar result was reported by ***Hesami et al. (2014)***[27]**.** The improvement of albumin level in the 4 weeks PRP-treated rats could reflect the possible positive effect of PRP on liver regeneration [33] and its antiapoptitic effect [34]**.** These results were further proved by histopathological examinations of livers of PRP-treated rats which showed oval cell proliferation in the liver. These cells are considered the Haemopoietic Progenitor Cell (HPC) in rodents and their presence indicate

hepatic regeneration. Similar results were reported by ***Makridakis et al. (2009)[33] and Murata et al. (2007) [35].*** The study of ***Meyer et al. (2015)[36]*** showed that following liver injury, endogenous platelets adhere to the endothelium, followed by their activation and granules release which activates the Akt and ERK (Extracellular Signal- Regulated Kinase) signaling pathways stimulating hepatocyte proliferation.

However, treatment of TAA intoxicated rats of the 6 weeks group with PRP resulted in a non-significant increase in albumin level as compared to their respective TAA group, which could be attributed to the late administration of PRP that could not successfully regenerate the liver cells with full synthetic ability.

Concerning the studied oxidative markers in the present study, both serum and hepatic MDA levels showed significant elevations in the TAA intoxicated rats of both 4 and 6 wks groups when compared to their respective control rats. These results came in accordance with ***Hesami et al. (2014)[27] and Luo et al. (2015)[37]*** who reported increased oxidative stress with hepatotoxicity**. *Bruck et al. (2004)[38]*** and ***Cruz et al. (2005)[39]*** stated that the chronic TAA administration induced liver cirrhosis and oxidative stress, this was further supported by the significant positive correlation between hepatic levels of MDA and serum levels of ALT and AST in the present study. ***Uskokovic-Markovic et al, (2007)[40]*** recorded that, TAA enhanced oxidative stress, with free radical– mediated damage to lipids, proteins and DNA.

On the other hand, hepatic glutathione peroxidase (GPx) levels were insignificantly different among the 6 studied groups, also, the plasma total antioxidant capacity levels (TAC) were insignificantly different among the 3 studied 4 weeks groups. TAC levels were significantly lower in the 6 weeks TAA intoxicated rats compared to their respective control rats. These results may reflect defective antioxidant enzymes that was more obvious in the 6 weeks group which may be attributed to longer period of study. After treatment with PRP in both 4 and 6 weeks rats**,** both serum and hepatic MDA were normalized compared to their controls, denoting successful decline of oxidative stress by PRP. Improvement of liver function tests and oxidative markers in PRP treated rats could be explained by the presence of multiple growth factors that were identified by ***Roubelakis et al. (2014)[41]***, the authors detected 33 factors that were classified into groups according to the main function. A group that regulates angiogenesis including PDGF-AA, PDGF-AB/BB, angiopoietin-1, angiopoietin-2, and others. Another group is related to tissue repair including thrombospondin-1, FGF-1, EGF, and others. A Third group associated with cell migration including TIMP-1, TIMP-4, MMP8 and MMP9***[41].***

As regards the lipid profile, the TAA rats of 4 weeks group showed significant depressions in their plasma TGs, TC and HDL-C when compared to their respective control rats reflecting an impairment of liver functions.

In the 6 weeks group, TGs and TC levels were significantly decreased in the TAA rats compared to their respective control rats but HDL and LDL cholesterol levels did not change. These findings were further supported by significant negative correlations between serum ALT, AST and MDA levels on one side and plasma TC, TGs and HDL levels on the other side.

These reductions in lipid profile agreed with the reported data in the studies of **Abbasi et al. (2012)[6]**, **Subhan et al. (2012)[7]**, **Janicko et al. (2013)[8]** and **Boemeke et al. (2015)[9]** , the authors recommended using lipid profile as a prognostic indicator for advanced liver disease with respect to cholesterol levels and its fractions reduction and the Child-Pugh score and the MELD score (Model for End Stage Liver Disease.

The liver is the only organ that synthesizes most TC in human body (about 70%), and it is the site for TC transformation and excretion, therefore its level is reduced in liver disease. The hepatocytes in liver diseases lack energy resulting in fatty acid oxidation and decline in TG levels [42].

Also, the liver controls the rate limiting step of lipoprotein metabolism by synthesizing their enzymes, thus regulating their mutual conversion and metabolism, the secretion of endogenous lipoproteins, absorbing and removing their metabolites through their receptors on the hepatocytes surface to maintain equilibrium of TC and TG metabolism [43].

Habib et al. (2005)[44] identified that patients that have lower HDL-cholesterol are more susceptible to undergo liver transplantation in a year or probability of death exceeding 60%, and thus the authors suggested that HDL-cholesterol works as a liver function test and as a prognostic indicator to viral and/or alcoholic cirrhotic patients.

After triglycerides are distributed, the remnant cholesterol in VLDL lipoprotein will be distributed to the tissues as LDL-cholesterol and if VLDL production and secretion is reduced, LDL-cholesterol will be also reduced, this was reported by studies that stated that carriers of hepatitis C virus had low values of TC and LDL-Cholesterol compared to uninfected patients [45; 46; 47].

These biochemical observations were further supported by the histopathological examination of rats' livers. Histologically, TAA injected rats` livers showed cirrhosis and marked fibrosis compared to the control rats. Also, prominence of hepatic lipid vacuoles which were previously reported by **Felmlee et al. (2013)[48]** who concluded that chronic liver diseased patients show a state of steatosis and hypocholesterolemia. These histopathological findings came in agreement with **Ahmed et al. (2012)** [49], who recorded that the liver sections of TAA-treated animals showed severe toxicity of hepatocytes characterized by centrilobular necrosis, apoptosis, scattered inflammation and fatty change. Persistent apoptosis is a feature of chronic liver diseases which eventually activates fibrogenesis resulting in cirrhosis of the liver [50].

In the present study, upon early treatment with PRP, the 4 weeks rats lipid profile did not show any significant change from untreated rats; a result that denotes failure of lipid metabolism recovery in this group, however, in the 6 weeks rats treated with PRP, TC level was normalized. Also, LDL cholesterol was significantly elevated which may reflect a partial improvement in the metabolic function of the liver in the 6 weeks treated rats .

These results could be explained by administration of PRP for 2 weeks without the hepatotoxicant in the 6 weeks late treated group which might have a beneficial impact on the lipid metabolism of the liver regressing the severity of chronic liver disease .

Treatment of rats with PRP, reversed the histopathogical TAA- induced changes in liver, observed through improvement in liver fibrosis with regeneration of hepatocytes through oval cell proliferation. Moreover, there is a decrease in inflammatory cell infiltration. Therefore, sections of PRP treated rats became nearly similar to the control liver. These results agreed with **Hesami et al. (2014)** [27].

The previously described antifibrotic and regenerative effects of PRP could be attributed to some members of the matrix metalloproteinases (MMPs),which are endopeptidases, that can degrade components of the extracellular matrix (ECM) [51], they can also regulate inflammation and immunological responses acting as cytokines [52] .

In the study of **Olle et al. (2006)**[53], MMP-9 deficient mice showed a delayed liver regeneration after 70% hepatectomy, MMP-8 which is expressed by PRP[41]**,** has antifibrotic function, since their over expression was associated with significant reduction in liver fibrosis [54]**.**

Moreover, tissue inhibitors of metallo-proteinases (TIMPs) are capable of regulating proteolytic activities of MMPs in tissues[51]**.** Fortunately, PRP also contains TIMPs for regulation of MMPs [41]**,** altogether, MMPs and TIMPs seem to have important roles in the preservation of liver homeostasis[55]**.**

In summary, in the present study PRP exerted noticeable biological and histopathological effects in both early and late PRP injection on the progression of hepatic dysfunction in experimental liver cirrhosis. The activated PRP, successfully released growth factors that hindered the hepatic destruction as noticed from the reduction of liver enzymes, an effect that could be attributed to the antiapoptotic [34], antifibrotic [56] effects, besides liver regeneration [33]**.**

However, regain of the liver synthetic ability by PRP is conflicting as albumin level was normalized in the 4weeks PRP group but not in the 6weeks PRP group while lipid profile was partially normalized in the 6weeks PRP group but not in the 4weeks PRP group.

Conclusion: The study revealed a beneficial effect of both early and late PRP injection on the progression of hepatic dysfunction in experimental liver cirrhosis. The activated PRP, successfully released growth factors that hindered the hepatic destruction as noticed from the reduction of liver enzymes, enhancement of oxidative stress, improvement of albumin which was significant in early treatment with PRP and lipid profile which was more evident in late treatment.

Conflict of interest:

No one of the authors has any conflict of interest.

REFERENCES:

1. Sánchez-Valle V, Chávez-Tapia NC, Uribe M, Méndez-Sánchez N (2012): Role of oxidative stress and molecular changes in liver fibrosis: a review. *Curr Med Chem.; 19:4850–4860.*
2. Ezhilarasan D, Sokal E, Karthikeyan S, Najimi M (2014): Plant derived antioxidants and antifibrotic drugs: past, present and future. *J. Coast Life Med.; 2:738–745.*
3. Murphy SL (2000): Deaths: final data for 1998. *Nat vital stats Rep.; 48:1-105*.
4. Guan YS, He Q (2013): A Current Update on the Rule of Alternative and Complementary Medicine in the Treatment of Liver Diseases. *Evidence-Based Complementary and Alternative Medicine*.
5. Halsted CH (2004): Nutrition and alcoholic liver disease. *24(3):289-304.*
6. Abbasi A, Bhutto AR, Butt N, Lal K, Munir SM (2012): Serum cholesterol could it be a sixth parameter of Child-Pugh scoring system in cirrhotics due to viral hepatitis?. *J Coll Physicians Surg Pak.*; *(8):484–487*.
7. Subhan F, Khan I, Arif R, Khan A, Khan A (2012): Serum lipid profile as an indicator of the severity of liver damage in cirrhotic patients. *Rawal Medical Journal; 37(4):387–389.*
8. Janicko M, Veselíny E, Lesko D, Jarcuska P (2013): Serum cholesterol is a significant and independent mortality predictor in liver cirrhosis patients. *Ann Hepatol.; 12:581–587.*
9. Boemeke L, Bassani L, Marroni CA, Bertaso C, Gottschall A (2015): Lipid profile in cirrhotic patients and its relation to clinical outcome, *Arq Bras Cir Dig.; 28(2):132–135*
10. Müller A, Machnik F, Zimmermann T, Schubert H (1988): Thioacetamide-induced cirrhosis-like liver lesions in rats--usefulness

and reliability of this animal model. *Exp Pathol.; 34:229–236.*

11. Akhtara T & Sheikh N (2013): An overview of thioacetamide-induced hepatotoxicity. *Toxin Reviews*; *32(3):43-46.*
12. Murata S, Ohkohchi N, Matsuo R, Ikeda O, Myronovych A, Hoshi R (2007): Platelets promote liver regeneration in early period after hepatectomy in mice. *World Journal of Surgery; 31(4):808–816.*
13. Shoeib HM, Keshk WA, Foda AM, Abo El Noeman SEAE (2018). A study on the regenerative effect of platelet-rich plasma on experimentally induced hepatic damage in albino rats. Can J Physiol Pharmacol. 2018 Jun;96(6):630-636.
14. Salem NA, Hamza A, Alnahdi H, Ayaz N. (2018): Biochemical and Molecular Mechanisms of Platelet-Rich Plasma in Ameliorating Liver Fibrosis Induced by Dimethylnitrosurea. Cell Physiol Biochem. 2018;47(6):2331-2339.
15. Fan S, Weng CF (2005): Co-administration of cyclosporine an alleviates thioacetamide-induced liver injury. *World J Gastroenterol.; 11(10):1411–1419*
16. Hamilton B, Tol JL, Knez W, Chalabi H (2012): Exercise and the platelet activator calcium chloride both influence the growth factor content of platelet-rich plasma (PRP): overlooked biochemical factors that could influence PRP treatment. *British journal of sports medicine, bjsports.*
17. Doumas B, Watson W, Ard , Biggs HG (1971): Albumin standards and the measurement of serum albumin with bromocresol green, *Clin Chim Acta.; 31:87.*
18. Doumas BT, Biggs HG (1972): Determination of serum albumin; standard methods of clinical chemistry, Acad. *Press NY.; 7:175.*
19. Bergmeyer HU, Horder AR, Rej R (1986): International Federation of Clinical Chemistry (IFCC) Scientific Committee, analytical section: approved recommendation 1985 on IFCC methods for the measurement of catalytic concentration of enzymes. Part 3. IFCC method for alanine aminotransferase (L-alanine: 2-oxoglutarate aminotransferase, EC2.6.1.2). *J Clin Biochem.; 24:481-495*.
20. Satoh K (1978): Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. *Clin Chim Acta.; 90:37-43*.
21. Ohkawa H, Ohishi N, Yagi K (1979): Assay of lipid peroxides in animal tissue by thiobarbituric acid reaction. *Anal Biochem.; 95:351–358.*
22. Rifai N, Bachorik PS, Albers JJ (1999): Lipids, lipoproteins and apolipoproteins In: Burtis CA, Ashwood ER, eds. Tietz textbook of clinical chemistry. 3rd ed. Philadelphia*: W.B Saunders Company; 809-861*.
23. Friedewald WJ, Levy RJ, Fredrickson DS (1972): Estimation of the concentration of low density lipoprotein cholesterol in plasma without the use of preparative ultracentrifuge. *Clin Chem.; 18:499.*
24. Koracevic D, Koracevic G, Djordjevic V, Andrejevic S, Cosic V (2001): Method for the measurement of antioxidant activity in human fluids. *J Clin Pathol.; 54(5):356-361*.
25. Paglia DE, Valentine WN (1967): Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med.; 70:158-169.*
26. Bancroft JD, Gamble M (2008): Theory and practice of histological techniques, sixth edition, *Churchill Livingstone Elsevier; 126, 150, 171, 601*.
27. Hesami Z, Jamshidzadeh A, Ayatollahi M, Geramizadeh B, Farshad O, Vahdati A (2014): Effect of Platelet-Rich Plasma on CCl4-Induced Chronic Liver Injury in Male Rats. *Int J Hepatol.*
28. Kew MC (2000): Serum aminotransferase concentration as evidence of hepatocellular damage. *Lancet; 355: 591–592.*
29. De David C, Rodrigues G, Bona S, Meurer L, González-Gallego J, Tun MJ, Marroni NP (2011): Role of quercetin in preventing thioacetamide-induced liver injury in rats. *Toxicol Pathol.; 39:949–957.*

30. Wolf PL (1999): Biochemical diagnosis of liver disease, *Indian Journal of Clinical Biochemistry; 14(1):59–90*.

31. Rao SV, Einstein JW, Das K (2014): Hepatoprotective and antioxidant activity of Lannea coromandelica Linn. on thioacetamide induced hepatotoxicity in rats. *International Letters of Natural Scinces; 3:30-43*.

32. Andersen MW, Ballal NR, Goldknopf IL, Busch H (1981): Protein A24 lyase activity in nucleoli of thioacetamide- treated rat liver releases histone 2A and ubiquitin from conjugated protein A24. *Biochem.; 20(5):1100-1104.*

33. Makridakis M, Gagos S, Petrolekas A, Roubelakis, MG, Bitsika V, Stravodimos K, Pavlakis K, Anagnou NP, Coleman J, Vlahou A (2009): Chromosomal and proteome analysis of a new T24-based cell line model for aggressive bladder cancer. *Proteomics.; 9:287–298.*

34. Singer AJ, Clark RA (1999): Cutaneous wound healing. *N Engl J Med.; 341:738–746.*

35. Murata S, Ohkohchi N, Matsuo R, Ikeda O, Myronovych A, Hoshi R (2007): Platelets promote liver regeneration in early period after hepatectomy in mice. *World Journal of Surgery; 31(4):808–816.*

36. Meyer J, Lejmi E, Fontana P, Morel P, Gonelle-Gispert C, Bühler L (2015): A focus on the role of platelets in liver regeneration: Do platelet-endothelial cell interactions initiate the regenerative process? *Journal of hepatology; 63(5):1263-1271.*

37. Luo M, Dong L, Li J, Wang Y, Shang B (2015): Protective effects of pentoxifylline on acute liver injury induced by thioacetamide in rats. *Int J Clin Exp Pathol.; 8(8):8990-8996*.

38. Bruck R, Schey R, Aeed H, Hochman A, Genina O, Pines M (2004): A protective effect of pyrrolidine dithiocarbamate in a rat model of liver cirrhosis. *Liver Int.; 24(2):169-176*.

39. Cruz A, Padillo FJ, Torres E, Navarrete CM, Muñoz-Castañeda JR, Caballero FJ, Briceño J, Marchal T, Túnez I, Montilla P, Pera C, Muntané J (2005): Melatonin prevents experimental liver cirrhosis induced by thioacetamide in rats. *J. Pineal Res.; 39(2):143-150*.

40. Uskoković-Marković S, Milenković M, Topić A, Kotur-Stevuljević J, Stefanović A, Antić-Stanković J (2007): Protective effects of tungsto-phosphoricacid and sodium tungstate on chemically induced liver necrosis in wistar rats. *J Pharm Pharm. Sci.; 10(3):340–349.*

41. Roubelakis MG, Trohatou O, Roubelakis A, Mili E, Kalaitzopoulos I, Papazoglou G, Pappa KI, Anagnou NP (2014): Platelet rich plasma (PRP) promotes fetal mesenchymal stem/stromal cell migration and wound healing process. *Stem Cell Rev.; 10(3):417-428*

42. Jiang M, Liu F, Xiong WJ, Zhong L, Xu W, Xu F, , Liu YB (2010): Combined MELD and blood lipid level in evaluating the prognosis of decompensated cirrhosis. *World J Gastroenterol.; 16(11):1397-1401*.

43. Zeng MD, Xiao SD (1997): Liver and endocrine. *Beijing; People's Medicial Publishing House*.

44. Habib A, Mihas AA, Abou-Assi SG, Williams LM, Gavis E, Pandak WM (2005): High-density lipoprotein cholesterol as an indicator of liver function and prognosis in noncholestatic cirrhotics. *Clin Gastroenterol Hepatol.; 3(3):286–291*.

45. Corey KE, Kane E, Munroe C, Barlow LL, Zheng H, Chung RT (2009): Hepatitis C virus infection and its clearance alter circulating lipids: Implications for long-term follow-up. *Hepatology; 50:1030–1037.*

46. Hsu CS, Liu CJ, Liu CH, Chen CL, Lai MY, Chen PJ (2008): Metabolic profiles in patients with chronic hepatitis C: a case-control study. *Hepatol Int.; 2:250–257.*

47. Lao XQ, Thompson A, McHutchison JG, McCarthy JJ (2011): Sex and age differences in lipid response to chronic infection with the hepatitis C virus in the United States National Health and Nutrition Examination Surveys. *J. Viral Hepat.; 18(8):571–579.*

48. Felmlee DJ, Hafirassou ML, Lefevre M, Baumert TF, Schuster C (2013): Hepatitis C virus, cholesterol and lipoproteins- impact for the viral life cycle and pathogenesis of liver disease. *Viruses; 5:1292–1324.*

49. Ahmed MK, Saleh ME, Sayed ME, Shalaby KA (2012): Anti-inflammatory effect of different propolis extracts in thioacetamide-induced hepatotoxicity in male rat. *J Basic Appl Sci.; 6:29-40.*

50. Malhi H, Gores GJ (2008): Cellular and molecular mechanisms of liver injury. *Gastroenterology; 134(6):1641-1654*.

51. Kessenbrock K, Plaks V, Werb Z (2010): Matrix metalloproteinases: regulators of the tumor microenvironment. *Cell; 141:52–67*.

52. Parks WC, Wilson CL, Lopez-Boado YS (2004): Matrix metalloproteinases as modulators of inflammation and innate immunity. *Nat Rev Immunol.; 4:617–629.*

53. Olle EW, Ren X, McClintock SD, Warner RL, Deogracias MP, Johnson KJ, Colletti LM (2006): Matrix metalloproteinase-9 is an important factor in hepatic regeneration after partial hepatectomy in mice. *Hepatology; 44:540–549.*

54. Endo H, Niioka M, Sugioka Y, Itoh J, Kameyama K, Okazaki I, Ala-Aho R, Kähäri VM, Watanabe T (2011): Matrix metalloproteinase-13 promotes recovery from experimental liver cirrhosis in rats. *Pathobiology; 78:239–252*.

55. Duarte S, Baber J, Fujii T, Coito AJ (2015): Matrix metalloproteinases in liver injury, repair and fibrosis. *Matrix Biol.; 147-156.*

56. Schmidt BA, Horsley V (2013): Intradermal adipocytesmediate fibroblast recruitment during skin wound healing. *Development; 140: 1517–1527.*

تأثير العلاج المبكر والمتأخر بالبلازما الغنية بالصفائح الدموية في أمراض الكبد المزمنة التجريبية: دور قياسات الدهون.

رانيا صلاح منصور، جيهان محمود حامد ، علياء محمد عبد المنعم، سهى محمد يسري

قسم الفسيولوجيا الطبية، كلية الطب جامعة عين شمس[1]

الخلفية: مرض الكبد المزمن مشكلة صحية عامة في مصر. مادة الثيوأسيتاميد يسبب ضررًا بالكبد يشبه تلف الكبد لدى الإنسان، ولذلك يتيح تقييم الطرق العلاجية الجديدة مثل البلازما الغنية بالصفائح الدموية. تم اقتراح قياسات الدهون كمؤشر تنبؤي في المرضى الذين يعانون من أمراض الكبد المتقدمة.

هدف البحث: تقييم تأثير العلاج المبكر والمتأخر بالبلازما الغنية بالصفائح الدموية على علامات إصابة الكبد بما في ذلك قياسات الدهون، في الفئران المصابة بأمراض الكبد المزمنة المستحدثة بالثيوأسيتاميد.

المواد والطرق: تم تقسيم 60 من إناث الجرذان إلى مجموعة ٤ أسابيع ومجموعة ٦ أسابيع، وتم تقسيم المجموعتين إلى ٣ مجموعات فرعية.

مجموعة فئران الثايو-أسيتاميد: تلقت سم الكبد مرتين أسبوعياً لمدة ٤ أسابيع في كل من المجموعتين ٤ و٦ أسابيع،

مجموعة فئران الثايو-أسيتاميد / المعالجة بالبلازما الغنية بالصفائح: تم إعطاء البلازما الغنية بالصفائح الدموية مباشرة بعد السم لمدة ٤ أسابيع (فى مجموعة العلاج المبكر- ٤ اسابيع) أو بدأت بعد أسبوعين من TAA ولمدة ٤ أسابيع (في مجموعة العلاج المتأخر- ٦ اسابيع)،

ومجموعة المراقبة: لم تتلق السم او العلاج

شمل التقييم الكيميائى قياس انزيم الالنين ترانس أمينيزو اسبارتيت ترانس أمينيز و المالوندالديهيد

و الالبيومين و السعة الكلية لمضادات الأكسدة و المالوندالديهيد و مستوى الدهون وتحديد مستوى كلا من المالوندالديهيد والجلوتاثيون بيروكسيدازفى الكبد

النتائج: أظهرت مجموعتا الثايو-أسيتاميد ارتفاعًا ذا دلالة احصائية في انزيم الالنين ترانس أمينيزو اسبارتيت ترانس أمينيزو المالوندالديهيد الكبدى و انخفاضا ذا دلالة احصائية في تركيز الالبيومين و مستوى الدهون.

أدى العلاج المبكر والمتأخر بالبلازما الغنية بالصفائح الدموية إلى تحسن كبير في إنزيمات الكبد و**المالوندالديهيد** فى الدم والكبد. تم تسوية الألبومين في مجموعة العلاج المبكر بينما تم تسوية الكوليسترول فى البلازما في مجموعة العلاج المتأخر.

الخلاصة: العلاج بالبلازما الغنية بالصفائح الدموية يقلل من إنزيمات الكبد ويحسن مستوى الدهون والألبومين وكذلك الإجهاد التأكسدي.