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RESEARCH ARTICLE

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Hepatoprotective effect of pomegranate peel (*Punica granatum* L) against thioacetamide-induced cirrhosis

ABSTRACT:

pomegranate peel is widely used in the Middle east as herbal medicine, its action as hepatoprotective agent still remains to be clarified. To understand its influence on liver vital functions, seventy-five male rats (aged 13 – 15 weeks) were divided into 15 categories randomly including 5 rats per category, and were injected with either Thioacetamide(TAA), or administered pomegranate peel (PP) plus injected TAA or administered selenium plus injected TAA or administered with 0.9 percent normal saline solution as a control. The hepatoprotective effect of herbs was evaluated by measuring levels of serum marker enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and measurement hepatocyte growth factor (HGF). The histological studies found that the supplementation of pomegranate peel significantly ($p < 0.05$) reduced the damaging effects of TAA on the liver. ALT, AST, and HGF levels declined in treatment group due to the hepatic injury induced. A comparative histopathological study of the liver sections different groups demonstrated that the pomegranate peel drives to normal liver architecture suggesting its use as hepatoprotective Alternative medication.

KEY WORDS:

Thioacetamide, pomegranate peel, Hepatocyte growth factor.

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INTRODUCTION:

Cirrhosis is an important reason of morbidity and death-rate in more developed countries. It's the fourth most common cause of death in central Europe; it outcomes in 1.3 million deaths for each year worldwide. During the few past years, many natural products and dietary component have been evaluated as potential chemo preventive agent. The natural products are a rich source of potentially therapeutic drugs, but many natural products must be structurally modified and optimized to become useful pharmacological agents.

Pomegranate peel has been widely known of the therapeutic potential in different civilizations. Especially in Egyptian civilization, several common diseases such as "inflammation, diarrhoea, intestinal worms, cough and infertility" have been treated by using pomegranate peel extract. In the last decade have been initiated intensive research into further research by the international scientific community to role of pomegranate peel in human health because the peel of these fruit from the compounds of anti-oxidation with medicinal qualities (Lansky and Newman, 2007).

The potential healing characteristics of PP are extensive-ranging and comprising prevention and treatment of cancer (Dikmen *et al.*, 2011), cardiovascular disease (Jurenka, 2008), diabetes and Other potential applications include infant brain ischemia, Alzheimer's disease (Middha *et al.*, 2012), dental conditions (Viuda-Martos *et al.*, 2010), and erectile dysfunction, male infertility, arthritis, obesity (Kanatt *et al.*, 2010), dermal wounds (Hayouni *et al.*, 2011).

MATERIAL AND METHODS:

Plant peel:

The dried plant peel of pomegranate was taken from local Iraqi herbal shops. They were ground into a fine for powder with a mechanical grinder before methanolic extraction by Soxhlet extraction device. The powdered plant peel was then passed across a fine sieve and stored in an airtight reservoir.

Experimental Animals:

Seventy-five male Wistar rats weighing obtained from the rats household collage of medicine, University of Baghdad were used in this experimental. The animals were housed in aplastic caged and were kept on standard Circumstances from temperature 25 ± 3ºC and humidity 50 – 60% and they provided with pellet food and tap water ad libitum and stayed at 12 h dark-light period. The caged were embedded with in wooden shelves in the animal household of collage of Science, University of Kufa. The rats were distributed into five groups, each of which with fifteen rats; each group was divided into three subgroups according to the treatment period (one month, two months and three month).

Preparation of Thioacetamide:

Thioacetamide (Sigma-Aldrich, Switzerland) was prepared freshly by diluting in sterile distilled water (2 ml/kg B.W.) and mixed well until all crystalline molecules were dissolved.

Experimental Protocol:

Seventy-five male rats were randomly divided into five groups, each of which with fifteen rats but each group divided into three subgroups according to the treatment period (one month, two months and three months): Group 1 (control group); rats were injected intraperitoneally with sterile distilled water (2 ml/kg) thrice weekly for (one month, two months and three months). Group 2 (hepatotoxic group); rats were administered orally with one dose intraperitoneally (ip) with 200 mg/kg of TAA thrice weekly for (one month, two months and three months). The injection protocol above was according to Alshawsh *et al*. (2011). Group 3; rats were orally administered pomegranate peel (400 mg/kg) daily and injected ip with 200 mg/kg of TAA thrice weekly for (one, two, and three months). Groups 4 rats; were administered orally with the pomegranate peel (200 mg/kg and injected ip with 200 mg/kg of TAA thrice weekly for (one, two, and three months). Groups 5; rats were orally administered selenium (200 mg/kg daily and injected ip with 200 mg/kg of TAA thrice weekly for (one, two, and three months).

Hepatic Biochemical Parameters:

Blood was collected from all rats and the serum was isolated to determine the enzymes of liver function such as alanine aminotransferase (ALT), aspartate aminotransferase (AST) by the ultraviolet spectrometry method, using Quick auto II. These were analysed using a Biochemical Autoanalyzer (AU5232; Olympus, Tokyo, Japan).

Histological studies:

The histological studies were also carried out to support the above parameters by using special stain Masson trichrome and using immunofluorescent staining to examination the distribution of a grin in liver.

Determination of Serum hepatocyte growth factor (HGF):

Elisa kits were used to detect the hepatocyte growth factor in serum of rats, it is supplied by Bioassay, United State of America.

RESULTS AND DISCUSSION:

Thioacetamide "TAA" is well known to be a hepatotoxic and carcinogenic compound in animals and most possibly in humans too, although no studies have shown these effects clearly. histopathological changes produced in liver cirrhosis model induced by TAA in rats are similar to those found in humans and animals and is taken into consideration as a good model (Laleman *et al*., 2006). In a previous study, has been used TA-induced hepatotoxicity to check the preventive effect of peel pomegranate on the events involved in the renewal of the liver. The results obtained in that study supply proof that PP when administered intravenously before the TA significantly reduces liver damage because the preventive with the crude extract in the model of thioacetamide-induced hepatotoxicity in rats diminished and delayed liver injury by 66% at 24 hours (Dyroff and Neal, 1983.).

In the control group, the livers showed normal architect. The cytoplasm of liver cells is intact, and the hepatic cells have –prominent nuclei, and the nucleoli and the liver central vein and sinusoidal spaces are normal. In the liver cirrhosis groups induced by injection thioacetamide 200 mg/kg, liver tissue damage established as proven by the existence of:

(A) collagen fibres within central vein wall and extending to the pericentral region,

(B) collagen fibres in region of portal triad with fibroblast proliferation and mononuclear inflammatory infiltrate

(C) central vein with peri-centric collagen deposition surrounded by liver cells exhibiting swelling and vacuolar degeneration with mononuclear inflammatory infiltrate, necrotic debris and collagen fibres in the wall of two adjacent central vein for one, two, and three months, respectively, while groups selenium treated 250 mg/kg.

(D) collagen fibres in the wall of central vein surrounded by liver cell plates with hepatocyte vacuolar degeneration and mononuclear inflammatory infiltrate.

(E) collagen fibres and fibroblast proliferation with mononuclear inflammatory infiltrate and hepatocyte necrosis.

(F) liver cells swelling and vacuolar degeneration for (1, 2, & 3 months), respectively. and groups pomegranate peel treated 200 mg/kg,

(G) particulate matter inside Kupffer cells and liver cell vacuolar degeneration.

(H) vacuolar degeneration, inflammatory cell and hepatocyte necrosis and mononuclear inflammatory infiltrate.

(I) just vacuolar degeneration for (1, 2, and 3 months), respectively. Also, groups pomegranate peel treated 400 mg/kg.

(J) portal triad surrounded by liver cell plates with particulate matter inside Kupffer cells.

K) region of portal triad with collagen fibres deposition surrounded by liver cell plates with hepatocyte vacuolar degeneration

(L) it is normal tissue for one, two, and three months, respectively (Fig. 1).

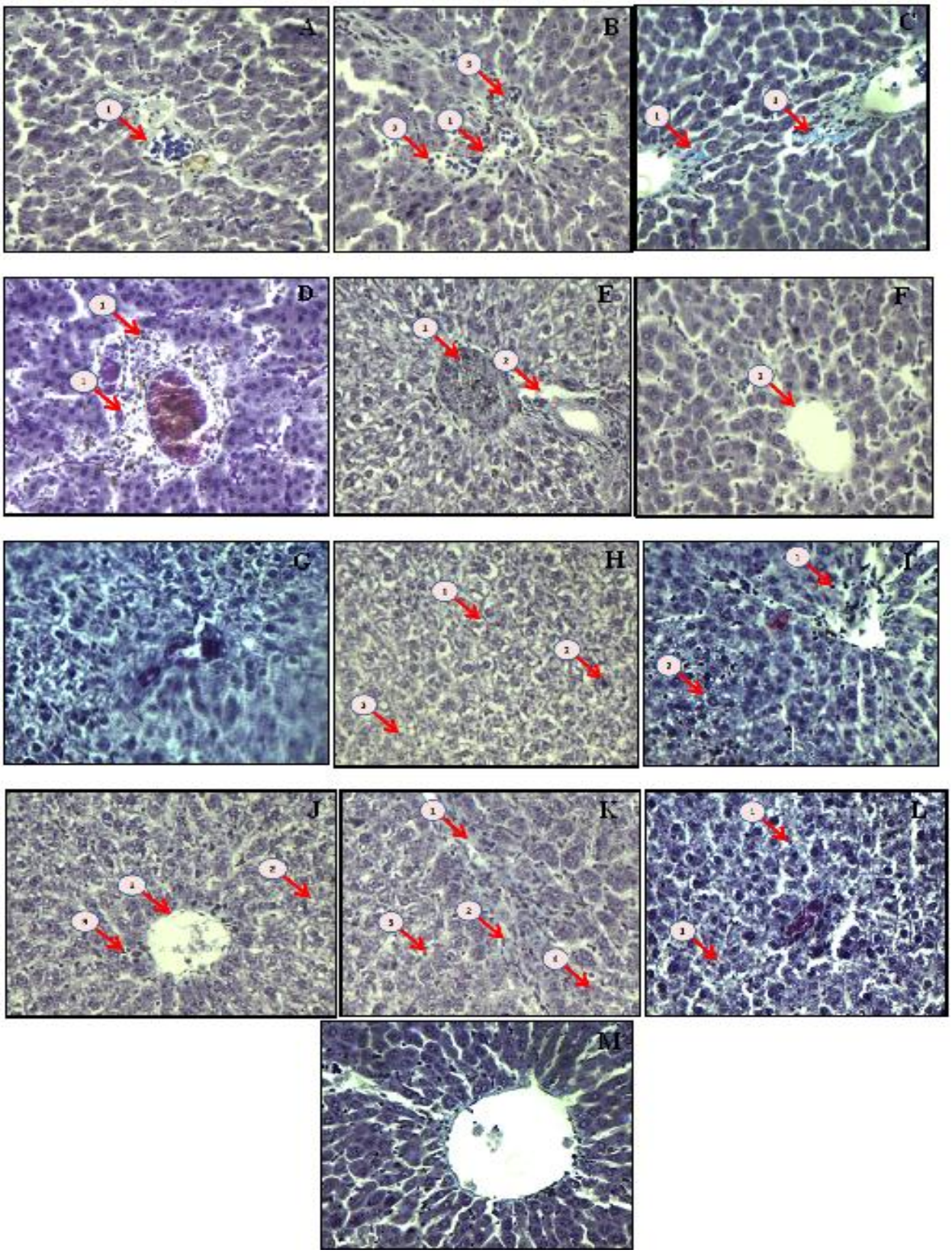

Fig. 1. Histopathological sections of livers sampled from rats in different experimental groups, thioacetamide alone and in combination with pomegranate peel or in combination with selenium on fibrosis grade, in male Wistar rats. (M) Negative control, (A, B, C) Thioacetamide treated, injection for (1, 2, & 3 months), respectively (D, E, & F) selenium treated (250 mg/kg), orally for (1, 2, & 3 months), respectively,(G,H,I) pomegranate peel treated (200 mg/kg), orally for (1, 2, & 3 months) respectively, (J, K, L) pomegranate peel treated (400 mg/kg), orally for (1, 2, & 3 months), respectively, (Masson trichome stain original magnification 20 x).

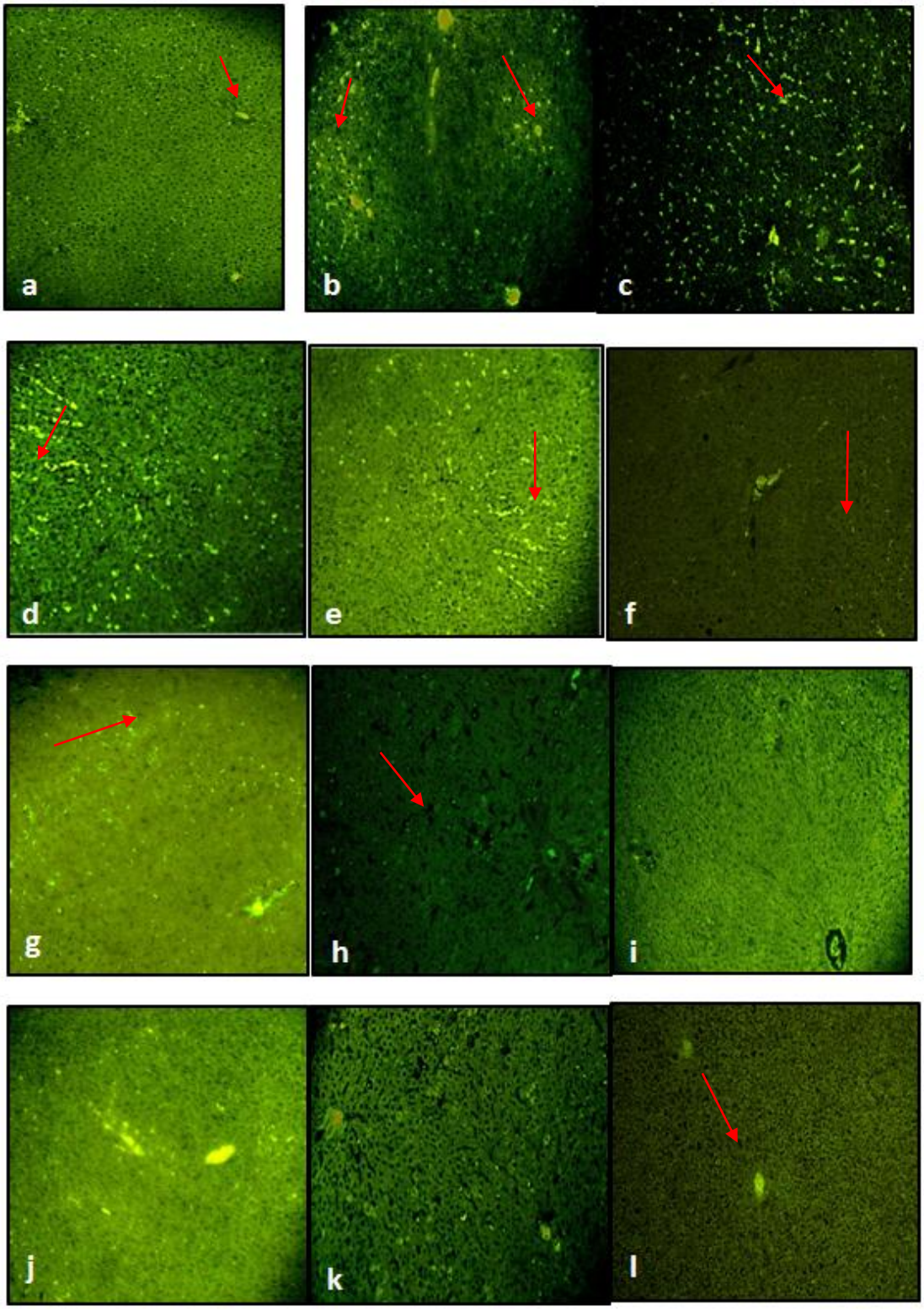

Fig. 2 Agrin immunostaining in liver cirrhosis in comparison with treatment with pomegranate peel (PP) or selenium. Basement membranes sinusoid are strongly Agrin-positive in groups treated with thioacetamide 200 mg/kg (a,b,c). Agrin immunostaining becomes weaker but persists in poorly differentiated in treating with selenium 250 mg/kg and PP 200 mg/kg, even in those with an atypical immunophenotype – arrow red- (d, e, f, g, h, & i). but is scarce or missing in treating with PP 400 mg/kg in liver (j,k,l).

Immunofluorescence study showed clearly that the Agrin were expressed in liver cirrhosis groups that involved male rat's injection by Thioacetamide 200 mg/kg, where its noted a gradual increase in the expression of Agrin from 1,2 to 3 months (Fig. 2). While the male rats that administration pomegranate peel (PP) with concentration 200 mg/kg and groups that demonstrated selenium 250 mg/kg (as commonly used drugs in the treatment of

cirrhosis) for one, two, and three months have shown a decrease in the expression of Agrin (Fig. 2). Also, the male rats that administration PP with concentration 400 mg/kg for one, two, and three months were shown a decrease in the expression of Agrin, which is a sign of cirrhosis of the liver, as if the extract of PP removed the effect in the sections of tissue (Fig 2).

The liver plays a key role in the metabolism of foreign materials that intervention its and it is the major organ responsible for many essential functions in the body. Through exposure to the environment, consumption of contaminated food or during exposure to chemical materials in the professional environment. All these compounds –outputs at various of toxic materials –to human beings– through– they expose to these compounds (Athar *et al*., 1997). despite the evolution of different therapeutic strategies for liver diseases, liver fibrosis, cirrhosis and its complications (liver failure, portal hypertension, and hepatocellular carcinoma) are not significantly reduced in many clinical cases (Mavier and Mallat, 1995). Thus, the high prevalence of liver diseases underscores the need for efficient and cost-effective treatment (Saller *et al*., 2001). Therefore, it is necessary to search for alternative medication for the treatment of liver diseases to substitute the currently used drugs of non-uncertain activity and safety.

Thioacetamide (TAA) has been considered a model to induce acute liver injury in rats for several years (Shapiro *et al*., 2006). Thioacetamide is a highly specific hepatotoxic compound causing liver injury and dysfunction and is known widely to stimulate hepatic– injury by proliferation of ROS —because containing thiono-sulfur compound (Wang *et al*., 2012). When hepatic cell membrane is deteriorated, the enzymes ALT, AST, and ALP which are normally existing in the cytosol, infiltrated into circulation from hepatocytes (Kirchain and Gill, 1997). TAA is known to make alterations in cell membrane permeability by its metabolite thioacetamide-S-oxide (Neal and Halpert, 1982) and the elevated serum enzymes are signal of cellular infiltration and lack of workable safety of the cell membrane of the liver (Drotman and Lawhorn, 1978).

As a result, the activity of ALT, AST, and ALP are raised in serum. Therefore, the activities of ALT, AST, and ALP are the most repeatedly used signals for liver diseases. In this study, administration of TAA in rats caused high increase in the activity of ALT and AST (Table 1). These findings are in good agreement with those of earlier workers who notify similar biochemical shifts (de David *et al.,* 2011).

Interestingly, treatment with *Punica granatum* peel methanolic extract for 12 weeks effectively ameliorated the significant elevation in serum ALT and AST ($P < 0.05$) as compared to the value of LSD between control and treatment group) and in TAA administered rats (Table 1). These results agree with those of El-Alfy *et al*. (2014) who found that administration of pomegranate peel extract significantly reduced the damaging impact of TAA on the liver.

The influence of *Punica granatum* on liver enzymes could be referred to the antioxidant effectiveness of its active compounds. Pomegranate juice, peel, seeds – all have a potent antioxidant efficiency due to their active compounds that are electron donors, which can interact with free radicals to transform them to more stable produces and finish radical series reaction (Singh *et al*., 2002). Pomegranate peel have very high total antioxidant activity because it has proportion of total "flavonoids and polyphenolic" compounds are high. Therefore, the effects antiapoptotic and antioxidant of PP are related to the effects of its polyphenols, such as "flavonoids epicatechin, epigallocatechin gallate, quercetin, luteolin and naringenin, phenolic acids chlorogenic and caffeic acids ellagitannin tannic acid and corilagin (Lansky and Newman, 2007).

Serum hepatocyte growth factor (HGF) was significantly increased in rats given TAA as compared to the negative control ones (Table 1). HGF play a significant role in liver proliferation as an endocrine or paracrine factor. HGF is made by non-parenchymal cells in the liver (Matsumoto and Nakamura, 1991). And it acts on each of the liver parenchymal cells and epithelial cells of the bile duct. The signal-transducing receptor for HGF is the c-met protooncogene produce of transmembrane tyrosine kinase (Naldini *et al*., 1991). HGF induces hepatic survival in various models of liver injury by stimulating liver regeneration and activating hepatoprotection. It has been proven that the major growth factors excreted after hepatic damage are HGF, 'transforming growth factor- α (TGF-α)" and "epidermal growth factor (EGF)"–. HGF is acts as a hepatotropic factor and the most potent mitogen for mature hepatocytes

The increased serum HGF level was restored to close normal levels in *Punica granatum* treated group as compared to the positive control group. This result agrees with that of Bassiouny *et al.* (2011) who reported that *Punica granatum* significantly reduce HGF levels in rats. This influence may be due to *Punica granatum* flavonoid level as Huang *et al*. (2012). The study concluded that pomegranate peels can be used as alternative medicine.

Table 1. Effect of PP ethanol extract on liver functions parameters and Hepatocyte growth factor at the three periods (one month, two months, and three months).

Periods / Treatment	HGF			ALT			AST		
	1 month	2 months	3 months	1 month	2 months	3 months	1 month	2 months	3 months
Control	952.132 ±11.806	965.130 ±11.249	967.490 ±6.803	67.372 ±2.197	66.220 ±1.916	66.260 ±2.528	15.580 ±.435	15.860 ±.725	15.900 ±1.099
TAA200 mg/kg	919.444 ±7.781	1024.412 ±18.877	1064.957 ±9.266	57.640 ±2.110	85.720 ±1.926	113.500 ±8.352	11.980 ±1.623	22.840 ±.795	30.100 ±2.142
Se 200 mg/kg	956.786 ±17.183	966.578 ±17.175	962.602 ±17.993	72.080 ±2.496	58.840 ±.482	61.120 ±1.455	21.300 ±1.244	19.240 ±2.201	15.720 ±1.403
PP 200 mg/kg	962.238 ±17.729	971.988 ±12.861	957.452 ±15.415	73.400 ±2.371	83.400 ±2.433	80.200 ±2.248	11.360 ±1.036	20.260 ±.629	26.820 ±2.180
PP 400 mg/kg	973.616 ±9.117	993.320 ±2.394	948.912 ±9.918	75.180 ±2.032	76.860 ±1.411	72.620 ±4.534	13.360 ±1.310	20.340 ±.450	19.340 ±1.015
LSD	78.830	81.283	74.249	13.261	10.396	26.892	7.059	6.779	9.710

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تأثير قشور الرمان الوقائي ضد تليف الكبد المستحدث بمادة الثايواستيمايد

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يستخدم قشر الرمان على نطاق واسع في الشرق الأوسط كدواء عشبي. ومع ذلك فإن تأثيره كأحد العوامل الكبدية المعزولة لا يزال بحاجة إلى توضيح اكثر، ولفهم تأثيره على الوظائف الحيوية للكبد، تم تقسيم خمسة وخمسين من ذكور الفئران تتراوح أعمارهم بين 13-15 أسبوعًا إلى 15 مجموعة عشوائية بواقع 5 جرذان لكل مجموعة، وتضمنت الدراسة معايير فسيولوجية ونسيجية لتقييم الدور العلاجي والوقائي لمستخلص قشور الرمان PP (200 و 400) ملغم/كغم من وزن الجسم اتجاه تليف الكبد المستحث thioacetamide(TAA) والمضاعفات الناتجة عنه مقارنة بمجموعة السيلينيوم selenium وذلك بإعطائه فمويا (250) ميكروغرام / كغم. وتضمنت مدة التجربة شهر وشهرين وثلاثة أشهر وتم حقنها إما TAA، أو PP المعززة بالإضافة إلى حقن TAA أو حقن السيلينيوم المحقون بالإضافة إلى حقن TAA أو باستخدام محلول ملحي طبيعي بنسبة 0.9 في المائة كمجموعة سيطرة. تم تقييم تؤثر الكبد من الأعشاب عن طريق قياس مستويات الأنزيمات الكبدية في المصل مثل الألانين ألانين (ALT) والألانين اسبارتاتي (AST) وعامل النمو الكبدية القياس (HGF). وقد أجريت الدراسات النسيجية أيضا لدعم المعلمات المذكورة أعلاه باستخدام صبغات خاصة مثل ماسون ترايكوم لملاحظة التغيرات النسيجية في الكبد. ووجدت الدراسة أن مكملات قشور الرمان بشكل كبير وتحت مستوى احتمالية ($P < 0.05$) قللت من التأثيرات الضارة على تليف الكبد المستحدث بواسطة TAA, حيث انخفضت مستويات ALT و AST و HGF في مجموعة المعاملات بالمقارنة مع مجموعة السيطرة واستنتجت الدراسة أن قشور الرمان يقود إلى هندسة الكبد وعمله بصورة طبيعية مما يوحي بأنه قد يستخدم كدواء بديل لعلاج التليف الكبدي.